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Use of Organic Acids for Controlling Damping-off Caused by *Rhizoctonia solani* on Cotton

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ABSTRACT

The four organic acids; Gallic acid (GA), Jasmonic acid (JA), Oxalic acid (OA) and Tannic acid (TA); (used in three concentrations for each acids) were evaluated *in vitro* and *in vivo* for suppression of *Rhizoctonia solani* caused damping-off on cotton. *In vitro* study showed that JA and OA were ineffective in inhibiting linear growth of the fungus, while GA and TA showed antifungal activities. In non-infested soil, none of the tested acids caused phytotoxicity under greenhouse conditions for seedlings of cotton cultivar Giza 95 when they were applied as soil drench. All the organic acids caused significant increases in plant height and dry weight of seedlings. In infested soil, GA was effective in controlling damping-off disease only at the low concentration (25.0 mM). All the applied concentrations of the other acids were effective in controlling the disease. Plant height and dry weight were not affected by any acid. The effects of organic acids on peroxidase, polyphenol oxidase, free phenolic compounds and total phenolic compounds were variable depending upon the applied concentration.

Keywords: Organic acids, *Rhizoctonia solani*, Damping-off, Cotton

INTRODUCTION

Rhizoctonia solani Kühn [(*Thanatephorus cucumeris*) (Frank) Donk] is a soil plant pathogen having worldwide distribution, a great ecological diversity and a vast host range. The pathogen is the most important soil borne fungus associated with cotton (*Gossypium barbadense* L.) damping off in Egypt (Moustafa *et al.*, 1993).

The pathogen usually attacks cotton seed or seedlings during germination and initial establishment of plants in the soil (Watkins, 1981). The use of seed dressing fungicides for controlling the disease is a common strategy. Rani *et al.* (2017) mentioned that fungicides affect adversely on soil fertility, soil microorganisms and other organisms like earthworms and even biological nitrogen fixing bacteria. Fungicides also cause soil and ground water pollution and have hazardous effect on human being who applies them. Moreover, use of fungicides led to development of fungicides resistant strains of the pathogen.

The phenomenon by which a plant's own defense mechanisms are induced due to prior treatment with either biological or chemical agent is known as Systemic Acquired Resistance (SAR) (Sticher *et al*., 1997). Induction of resistance in plants to overcome pathogen infection by using some chemicals is a promising approach for controlling plant diseases (El-Mohamedy *et al*., 2014).

Many studies have been conducted on chemical resistance inducers used for controlling root rot under greenhouse and field conditions (El-Mougy *et al.*, 2004; and El-Mohamedy *et al.*, 2014).

No doubt these chemicals are environmentally safe, economically cheaper and easy to apply with much less hazards comparing with conventional fungicides (Hassan *et al.,* 2007).

Jasmonic acid has been found to increase plant tolerance to variety of biotic and abiotic stresses including fungal pathogens (Fugate *et al.,* 2017).

Tannic acid occurs widely in root exudates, decaying plant residues and soil. It served as an ecological allelochemical, repressing the growth of the pathogen (Wu *et al.,* 2010).

Gallic acid seems to have antifungal activity against *Fusarium semitectum*, *F. fusiformis* and *Alternaria altternata* (Al-Zahrani, 2012).

Oxalic acid (OA) is known to induce high level of systemic resistance in oil seed against *Sclerotinia sclerotiorum* (Toal and Jones 1999). Exogenous application of OA caused induction of resistance in rice plants against *R. solani* infection (Jayaraj *et al.*, 2010).

The host-pathogen interaction induces signaling molecule in plants system, which lead to production of antimicrobial compound. Defensive enzymes are among the most influential and widely distributed products in the plants. Peroxidase and polyphenol oxidase were reported in plants treated with various biotic and abiotic inducers (Raghvendra *et al.*, 2007). The role of oxidative enzymes such as peroxidase and polyphenol oxidase could be explained as anoxidation process of phenol compounds to oxidized products which may limit the fungal growth (Nawar and Kuti, 2003). The chemical inducers might stimulate some defense mechanisms such as phenolic compounds, oxidative enzymes (Amel *et al.,* 2010; and El-Mohamedy *et al*., 2014).

The objective of the present study was to investigate the effects of some organic acids on protection of cotton (*Gossypium barbadense* L.) cultivar Giza 95 against damping-off disease caused by *Rhizoctonia solani* under greenhouse conditions and to detect the impacts of the tested acids on some defense mechanisms (oxidative enzymes and phenolic compounds) of cotton against infection.

MATERIALS AND METHODS

Source of fungal pathogen:

Rhizctonia solani isolate used in this study was obtained from the fungal collection of Cotton and Fiber Crops Dis. Res. Sec., Plant Pathology Res. Inst. Agric. Res. Center. The isolate was originally isolated from cotton seedling infected with damping-off.

Effect of organic acids on fungal linear growth:

In vitro antifungal assays of the organic acids against the fungus were performed with the poisoned plate technique as described by (Adawy *et al.,* 2018).

Effect of organic acids on growth of cotton cultivar Giza 95 under greenhouse conditions:

Autoclaved clay soil was dispended in sterilized 15-cm diameter clay pots and planted with 10 seeds of cotton cultivar Giza 95. The tested acids solutions were added to the pots (50 ml/ pot) at three replicates for each concentration and after forty days stand, plant height and dry weight were recorded (Table 2). Random samples of cotton seedlings were collected from each treatment and used for further biochemical studies.

Effect of organic acids on damping-off incidence under greenhouse conditions:

Substrate for growth of *R. solani* isolate was prepared in 50 ml flasks, each flask contained 15 gm of sorghum grains and 25 ml of water. Contents of flasks were autoclaved for 30 min. The autoclaved flasks were inoculated with fungal growth taken from one-week old culture on Potato Dextrose Agar medium (PDA) plates and allowed to colonize sorghum for 7 days. Autoclaved clay soil was infested with the isolate of *R. solani* at the rate of 1.0g/kg soil. Infested soil was dispended in sterilized 15-cm diameter clay pots and planted with 10 seeds of cotton cultivar Giza 95 with three replicates for each treatment. Three replicates contained autoclaved soil served as control. Solution of each concentration (50 ml/pot) of each tested acid was added to each replicate (Table 3). After forty days disease incidence, plant height and dry weight of plants were recorded. Random samples of cotton seedlings were collected from each treatment and used for further biochemical studies.

Preparation of enzyme extracts and the assay methods

Crude enzyme extracts for the assays were prepared according to (Aluko and Ogbadu, 1986). The Enzymes activity was assayed using spectrophotometer (spectronic 106).

Peroxidase enzyme assays

The Peroxidase activity was measured in different samples as described by (Worthington, 1972) as follows: An amount of 0.5ml mL diluted extracts was mixed with 1.5 ml of phosphate buffer pyrogallol (pH 6.0), after mixing; the solution was added 1.1ml H2O2 30%. The change absorbance of the reaction mixtures was measured against blank at 430 nm wavelength (Δ430).

Polyphenol oxidase enzyme assays

Polyphenol oxidase activity was measured following the method described by (Esterbaner *et al*., 1977). The enzyme extract (0.5 mL) was mixed with 2.2 ml of 0.1 M phosphate buffer. After the sample was adjusted to zero absorbance, 0.5 ml of 0.01 M catechol in 0.1 M phosphate buffer was added to the mixture and the volume was made up to 3 mL with distilled water. The reaction was quickly mixed and after the change in absorbance per minute was measured at 495 nm (Δ495).

Determination of phenolic compounds

The total and free phenols of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by (Simons and Ross, 1971). Sample and standard readings were made using a spectrophotometer (spectronic 106 spectrophotometer) at 520 nm against the reagent blank.

Statistical analysis

The obtained data were statistically analyzed by MSTAT-C software. The mean differences were compared by least significant difference test (LSD) at p ≤ 0.05.

RESULTS

Effect of the organic acids on linear growth of *R. solani* on PDA medium:

Table (1) showed that GA caused significant inhibition (57.44%) on linear growth only at the high concentration (105.0mM). Each of JA and OA did not cause inhibition of *R. solani* linear growth at all concentration. In contrast, TA caused significant inhibition 68.11%, 55.89% and 47.44% on linear growth at all used concentrations in comparison with the control.

Table 1. Effect of organic acids on linear growth of *R. solani* on PDA medium

Organic Acid	Applied Concentration(Mm)	Linear Growth(cm)	Inhibition (%)
Control	--	9.00[a]	
Gallic acid	105.00	3.83	57.44[b]
	55.00	8.67	3.67
	25.00	9.00	0.00
Jasmonicacid	0.20	9.00	0.00
	0.10	9.00	0.00
	0.05	9.00	0.00
Oxalic acid	2.00	9.00	0.00
	1.00	9.00	0.00
	0.50	9.00	0.00
Tannic acid	20.00	2.87	68.11
	10.00	3.97	55.89
	5.00	4.73	47.44

[a] Mean of three replicates
[b] Inhibition%= (control-treatment /control) x100
LSD (p ≤ 0.05) =0.41

Effect of organic acids on cotton seedlings grown in non-infested soil:

Data in Table (2) showed that the tested acids had no significant effects on percentage of seedling stand compared with control, while all treatments significantly increased each of the plant height and dry weight of seedlings compared with control. None of the tested acids showed phytotoxicity on seedlings of cotton cultivar Giza 95.

Table 2. Effect of organic acids on cotton seedlings (cv. Giza 95) grown in non-infested soil

Organic Acid	Applied Concentration (Mm)	Stand (%)	Plant height (cm/plant)	Dry weight (gm/plant)
Control	--	86.70[a]	14.89	0.085
Gallic acid	105.00	73.30	19.40	0.190
	55.00	73.30	20.67	0.175
	25.00	66.70	22.40	0.249
Jasmonicacid	0.20	86.70	20.32	0.197
	0.10	93.30	22.00	0.214
	0.05	86.70	20.10	0.205
Oxalic acid	2.00	73.30	22.20	0.262
	1.00	80.00	20.74	0.236
	0.50	80.00	24.33	0.325
Tannic acid	20.00	73.33	26.14	0.338
	10.00	86.70	21.67	0.268
	5.00	96.70	22.53	0.328
LSD p≤0.05)	--	N.S.	4.08	0.05

[a]Mean of three replicates

Effect of organic acids on cotton seedlings (cv. Giza 95) grown in infested soil with *R. solani*:

Data in Table (3) showed that *R. solani* caused significant decrease (43.33%) in seedling survival compared with non-infested soil (86.67%). The fungicide (Moncut) caused significant increase in seedling survival (93.33%) compared with seedling survival in infested soil. Gallic acid caused significant decrease (20.0 %) in seedling survival when it used by high concentration (105.0mM) and medium concentration (55.0mM), while it increased (53.33%) the survived seedlings at low concentration (25.0mM), but this increase was not significant. All the other tested acids caused significant increases in seedling survival at all concentration except JA at the low concentration (0.05mM) and OA at high concentration (2.0mM) where the increases were not significant. Data in Table (3) also showed that all tested acids at all used concentrations did not cause significant effects on plant height or dry weight.

Effect of organic acids on oxidative enzymes in cotton seedlings grown in non-infested soil:

Data in Table (4) showed that GA caused significant decrease (0.127) in peroxidase at high concentration (105.0Mm) compared with untreated control (0.704), while it caused significant increases in peroxidase at medium (55.0Mm) and low (25.0Mm) concentrations (1.626 and 0.890, respectively). The same result showed with OA. Jasmonic acid caused significant decreases in peroxidase activity at all used concentrations. In contrary, TA caused significant increases in peroxidase activity at all applied concentrations. Polyphenol oxidase activity significantly decreased under effect of application all tested acids except JA at the high concentration (0.20 mM) and TA at medium concentration (10.0mM) as it significantly increased (0.118 and 0.106, respectively) compared with untreated control (0.065).

Table 3. Effect of organic acids on cotton seedlings (cv. Giza 95) grown in soil infested with *R. solani*

Organic Acid	Applied Concentration (mM)	Surviva (%)	Plant height (cm/plant)	Dry weight (gm/plant)
Non-infested control	--	86.67[a]	14.89	0.085
Infested control	--	43.33	17.00	0.177
Fungicide(Moncut)	--	93.33	17.97	0.176
Gallic acid	105.00	20.00	17.83	0.148
	55.00	20.00	16.00	0.093
	25.00	53.33	15.89	0.111
Jasmonic acid	0.20	80.00	15.52	0.110
	0.10	80.00	16.37	0.179
	0.05	53.33	19.67	0.202
Oxalic acid	2.00	53.33	18.72	0.192
	1.00	93.33	17.50	0.143
	0.50	73.33	19.67	0.195
Tannic acid	20.00	86.67	20.00	0.170
	10.00	80.00	18.00	0.165
	5.00	80.00	19.00	0.171
LSD ($p \leq 0.05$)	--	19.7	N.S.	N.S.

[a]Mean of three replicates

Table 4. Effect of organic acids on oxidative enzymes in cotton seedlings (cv. Giza 95) grown in non-infested soil.

Organic Acids	Applied Concentration (Mm)	Peroxidase Activity (Δ_{430})	Polyphenol oxidase Activity(Δ_{495})
Control	--	0.704[a]	0.065
Gallic acid	105.00	0.127	0.033
	55.00	1.626	0.020
	25.00	0.890	0.024
Jasmonic acid	0.20	0.296	0.118
	0.10	0.459	0.057
	0.05	0.249	0.0215
Oxalic acid	2.00	0.322	0.030
	1.00	0.797	0.046
	0.50	0.909	0.0211
Tannic acid	20.00	1.050	0.030
	10.00	0.878	0.106
	5.00	1.266	0.019
LSD($p \leq 0.05$)	--	0.06	0.005

[a] Mean of three replicates

Effect of organic acids on phenolic compounds in seedling grown in non-infested soil:

Regarding Table (5), total phenolic compounds significantly increased by using GA only at high concentration (105.0Mm), OA at high (2.0Mm) and low (0.5Mm) concentrations (5.609, 5.933 and 6.142, respectively) compared with untreated seedlings (4.855). Tannic acid caused significant increase in total phenolic compounds at all used concentrations compared with untreated seedlings, while JA caused significant decrease in total phenolic compounds. On the other hand, free phenolic compounds significantly increased by application OA at low concentration (0.5Mm) and at all TA concentrations compared with control (4.72).

Table 5. Effect of organic acids on phenolic compounds in cotton seedlings (cv. Giza 95) grown in non-infested soil.

Organic Acids	Applied Concentration (Mm)	Total Phenolic Compounds (mg/g fw)	Free Phenolic Compounds (mg/g fw*)
Control	--	4.855[a]	4.702
Gallic acid	105.00	5.609	2.437
	55.00	4.409	3.293
	25.00	4.035	2.854
Jasmonic acid	0.20	4.409	3.645
	0.10	4.625	3.680
	0.05	4.424	4.026
Oxalic acid	2.00	5.933	4.903
	1.00	4.596	4.155
	0.50	6.142	5.636
Tannic acid	20.00	6.372	5.659
	10.00	7.206	5.155
	5.00	6.817	5.787
LSD($p \leq 0.05$)	--	0.25	0.29

[a] Mean of three replicates fw = fresh weight

Effect of organic acids on oxidative enzymes in cotton seedling grown in infested soil with *R. solani*:

Table (6) showed that peroxidase activity increased significantly (0.846) in seedlings grown in infested soil compared with seedlings grown in non-infested soil (0.704). The fungicide treatment caused significant increase in peroxidase activity compared with infested control. All treatments caused significant decrease in peroxidase except three treatments which were GA at medium concentration, OA also at medium concentration and TA at high concentration.

Data in Table (6) showed that polyphenol oxidase significantly decreased (0.053) in plants grown in infested soil compared with non-infested control (0.065). All treatments significantly decreased polyphenol oxidase compared with infested control except GA at low concentration (0.09), JA at medium concentration (0.066) and TA at all used concentrations compared with infested control (0.53).

Table 6. Effect of organic acids on oxidative enzymes in cotton seedlings (cv. Giza 95) grown in soil infested with *R. solani*.

Organic Acids	Applied Concentration (Mm)	Peroxidase Activity (Δ_{430})	Polyphenol oxidase Activity (Δ_{495})
Non-infested control	--	0.704[a]	0.065
Infested control	--	0.846	0.053
Fungicide(Moncut)	--	2.495	0.020
Gallic acid	105.00	0.155	0.040
	55.00	2.199	0.027
	25.00	0.303	0.090
Jasmonic acid	0.20	0.502	0.034
	0.10	0.246	0.066
	0.05	0.407	0.023
Oxalic acid	2.00	0.267	0.052
	1.00	1.449	0.026
	0.50	0.682	0.034
Tannic acid	20.00	1.223	0.054
	10.00	0.090	0.115
	5.00	0.574	0.057
LSD ($p\leq0.05$)	--	0.08	0.001

[a]**Mean of three replicates.**

Effect of organic acids on phenolic compounds in seedlings grown in infested soil with *R. solani*:

Regarding Table (7), total phenolic compounds significantly increased (5.524) in seedlings as a result of *R. solani* infection, compared with seedlings in non-infested soil (4.855). Moncut also caused significant increase (6.796) in total phenolic compounds compared with infested control (5.524). Gallic acid caused significant increase in total phenols at high and medium concentrations (6.868 and 5.962, respectively), while it caused significant decrease (4.452) in total phenols at the low concentration. Jasmonic acid caused increase (5.861) in total phenolic compounds only at the low concentration. Oxalic acid caused significant increase (6.048) in total phenolic compounds at the low concentration. Tannic acid caused increase (5.811) and significant increase (6.271) in total phenolic compounds at the high and low concentrations, respectively.

Presence of *R. solani* in the soil caused significant increase (5.219) in free phenolic compounds in infected seedlings compared with non-infested control (4.702). All treatments caused decrease or significant decrease in free phenolic compounds compared with infested control except the high concentration of TA which caused increase (5.600) in free phenolic compounds.

Table 7. Effect of organic acids on phenolic compounds in cotton seedlings (cv. Giza 95) grown in soil infested with *R. solani*.

Organic Acids	Applied Concentration (Mm)	Total Phenolic Compounds mg/g fw	Free Phenolic Compounds mg/g fw*
Non-infested control	--	4.855[a]	4.702
Infested control	--	5.524	5.219
Fungicide(Moncut)	--	6.796	5.241
Gallic acid	105.00	6.868	4.457
	55.00	5.962	4.946
	25.00	4.452	3.989
Jasmonic acid	0.20	5.085	4.299
	0.10	4.539	4.321
	0.05	5.861	4.357
Oxalic acid	2.00	5.495	4.709
	1.00	4.279	4.242
	0.50	6.048	4.321
Tannic acid	20.00	5.811	5.600
	10.00	4.754	4.091
	5.00	6.271	5.198
LSD ($p\leq0.05$)	--	0.421	0.48

[a]**Mean of three replicates** **fw = fresh weight**

DISCUSSION

The antifungal activity of organic acids as we have demonstrated in the present study is in agreement with some previous studies. For example, organic acids play a role for biological soil disinfestations as they suppress the growth of soil pathogens like *Fusarium* and *Rhizoctonia* (Noriaki*et al.,* 2006). Zaidi *et al.* (2008) found a significant inhibiting effect by the TA and GA on the growth of *Pectobacterium chrysanhemi*. They conducted that TA and GA had biological important effects, as antibacterial, antiviral and antifungal products. Hathout *et al.,* (2010) found that application of JA as seed presoaking treatment induced systemic resistance of bean plants against *R. solani*. Jayaraj *et al.,* (2010) found that pretreatment of rice plants with OA significantly reduced subsequent infection by *R. solani*. Wu *et al.,* (2010) found that TA decreased the growth and conidial germination of *Fusarium oxysporum* f. sp. *niveum*.

Organic acids exert their antifungal activities through a variety of mechanisms. Jasmonic acid (JA) is a naturally occurring hormone that functions as signaling compound for the induction of native plant defense responses (Dar *et al.*, 2015). Jasmonic acid is thought to protect by promoting the synthesis of proteins and metabolites which are toxic, harmful, or anti-nutritive to plant pathogens (Sun *et al.,* 2013). Tannin is a substance that can inhibit the enzyme activities released by *R. solani* and thus potential to be used as antifungal against the fungal species (Achmad *et al.,* 2015). This report lend support to our results which indicate that TA was the most effective acid in suppression *R. solani* damping-off under greenhouse conditions.

Organic acids may alter many parts of the cell and they have an effect on the respiration of the cell (Omar *et al.,* 2018).

The antimicrobial activity of some of the tested organic acids in the present study could be attributed to their stimulation of oxidative enzymes and phenolic compounds. The chemical inducers might stimulate some defense mechanisms such as oxidative enzymes and phenolic compounds (Amel *et al.,* 2010 and El-Mohamedy *et al.,* 2014). Lattanzia *et al*., (2006) mentioned that the highly toxic quinones which produced as a result of oxidization phenols by peroxidase and polyphenol oxidase inhibit fungal germination.

Anand *et al*., (2009) and Gogoi *et al*., (2001) reported that the increase in enzymatic activity of peroxidase and polyphenol oxidase stimulate an extra production and piling up of phenolics, which might prevent the pathogen to extend from the infected cells into the uninfected ones, and thus the infection can be suppressed or limited.

The toxic phenolic compounds in plant cells impact through the structure of bond form with cell wall components of plant tissues, encourage host resistant by inducing host defense mechanisms, prohibit the extent of fungal growth in plant tissues and penetrate the microorganisms and cause great damage to the cell metabolisms (Mahadevan and Sridhar, 1986; Subba Rao *et al*., 1988; Soni *et al*., 1992; and Kalaichelvan and Elangovan, 1995).

In general, the effects of organic acids on peroxidase, polyphenol oxidase, free phenolic compounds and total phenolic compounds were variable depending upon the applied concentration.

The results of the present study showed that the control of cotton damping-off by GA and TA, particularly TA could be attributed to the two mechanisms of antifungal activity and induced resistance. On the other hand, suppression of the disease by JA and OA was attributed mainly to their induction of Giza 95 resistance. This result indicates that the exact mode of action of controlling damping-off by organic acids depends upon the applied concentration.

The present study is a preliminary work on the efficiency of organic acids in controlling damping-off of cotton seedlings. In the future our attention should focus on field evaluation of organic acids by using a collection of commercial cotton cultivars.

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استخدام الأحماض العضوية فى مقاومة مرض موت البادرات الناجم عن الاصابة بفطر الريزوكتونيا سولانى على القطن

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فى هذه الدراسه قيمت اربعة أحماض عضوية هى حمض الجاليك وحمض الجاسمونيك وحمض الأوكساليك وحمض التانيك) باستخدام ثلاث تركيزات لكل حامض (من حيث قدرتها على تثبيط فطر الريزوكتونيا سولانى معمليا وعلى نباتات القطن تحت ظروف الصوبة .أظهرت النتائج فى المعمل عدم قدرة كل من حمض الجاسمونيك وحمض الاوكساليك على تثبيط نموالفطر على الأطباق بينما سجل كل من حمض الجاليك وحمض التانيك نشاط مضاد للفطر .لم يكن لأى من الأحماض العضوية المختبرة أى تأثير سام على بادرات القطن صنف جيزة 95 تحت ظروف الصوبة وذلك عند اضافتها للتربة الغير ملوثة بالفطرفى صورة محلول .بينما إضافة جميع الأحماض العضوية أدت الى زيادات معنوية فى أطوال البادرات والوزن الجاف لها .أما تحت ظروف التربة الملوثة بالفطر، فقد كان حمض الجاليك فعال فى مقاومة المرض عند استعماله فقط بالتركيز المنخفض) 25 مللى مول .(وكانت جميع الأحماض العضوية فعالة فى مقاومة المرض بجميع التركيزات المستعملة .لم يتأثر كل من أطوال البادرات واوزانها الجافة بأى من الأحماض العضوية المستخدمة تحت ظروف العدوى بالفطر .تباينت تأثيرات الأحماض العضوية المختبرة على كل من انزيم البيروكسيديز وانزيم البوليفينول اوكسيديز والمركبات الفينولية الحرة والمركبات الفينولية الكلية على البادرات السليمة والبادرات المصابة بناءً على التركيزات المستخدمة من هذه الأحماض العضويه.