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ACETATE ION IS AN INHIBITOR FOR SOME SOIL BORN PLANT PATHOGENIC FUNGI

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ABSTRACT

Acetate ion was found to have an inhibitory effect against three of sclerotia forming soil born plant pathogenic fungi *Sclerotium rolfsii, Sclerotium cepivorum* and *Sclerotinia sclerotiorum*. It showed no effect on other tested four plant pathogenic fungi *Fusarium solani, Rhizoctonia solani, Fusarium oxysporum* and *Macrophomina sp*. The inhibition of fungal growth was obtained at acetate ion concentration of 0.015 molar for *S. rolfsii* and at 0.03 molar for both *S. cepivorum* and *S. sclerotiorum*. Killing of grown fungi were achieved after treatment by 0.36 molar of acetate ion for one hour only for *S. rolfsii* and 0.74 molar for both *S. cepivorum* and *S. sclerotiorum*. Sclerotia of both *S. rolfsii and S. cepivorum* were killed after treatment by 0.74 molar of acetate ion for one hour only; while the sclerotia of *S. sclerotiorum* can not be killed by this method although using high concentrations acetate ion up to 1.5 molar and long time of treatment up to 24 hours. Other carboxylic acid salts (ammonium oxalate and ammonium citrate) showed no inhibition effect against any of the three fungi.

INTRODUCTION

Soil born plant pathogenic fungi *Sclerotium rolfsii, Sclerotium cepivorum* and *Sclerotinia sclerotiorum* causes white rot of stems and roots for several plants worldwide. These fungi survive in the soil as sclerotia, which are hardened structures of fungal mycelium (Ben Yephet *et al.,* 1993).

Sclerotium rolfsii is a devastating plant pathogen infecting over 500 plant species in 100 families (Punja, 1985; Punja, 1988; Sarma *et al*., 2002 and Azhar *et al*., 2003). *S. rolfsii* attacks diverse phases of the development of its hosts. It is able to survive and thrive within a wide range of environmental conditions. Growth is possible within a

broad pH range (5-8). The mycelium growth occurs between 20 and 35 °C (Azhar *et al*., 2003).

Sclerotium cepivorum causes the disease white rot on several *Allium* species. White rot of onion (*Allium cepa* L.) is a serious disease of onion worldwide (Jesus Recardo *et al;* 2002, Earnshaw *et al.,* 2000; Yong *et al.,* 2004 and Ulacio *et al.,* 2006).

S. sclerotiorum is an aggressive fungal plant pathogen that is known to attack over 360 species of host plants worldwide in many different soil types and environmental conditions (Dillard and Cobb, 1995). *S. sclerotiorum* causes diseases in snap beans, dry beans, cabbage, carrots, alfalfa, soybeans, lettuce, potatoes, sunflower and other host crops (Boland and Hall 1994 and Itamar *et al.,* 2006). The sclerotia germinate under the appropriate environmental conditions to produce fruiting bodies call apothecia (Abawi and Grogan, 1979). The apothecia produce ascospores that may infect susceptible plant tissue.

During a previous study, Abdel Azeiz *et al.,* (2007) isolated a bacterium (identified as *Bacillus psychrosaccharolyticus*) from Egyptian soil which able to inhibit the plant pathogenic fungus *Sclerotium rolfsii*. The inhibitory compound which is produced by this bacterium was separated and identified as a glycolipoprotein. Effect of different carbon and nitrogen sources on production of this compound by the isolated bacterium was studied. Ammonium acetate was tested among the tested nitrogen sources. The authors noticed that the culture filtrate of this bacterium, which had been grown on ammonium acetate as a nitrogen source, showed the maximum inhibition of *S. rolfsii* (95%) in spite of the lower concentration of the inhibitor glycolipoprotein as shown from the HPLC analysis. Therefore we suggested that the inhibitory effect may be due to one of these reasons: 1) Ammonium acetate enhanced production of other inhibitor by this bacterium against *S. rolfsii* instead of the inhibitor glycolipoprotein. 2) The ammonium acetate reacted with other component in the media such as glucose at the high temperature during autoclaving to produce an inhibitory compound. 3) Ammonium acetate itself has an inhibitory effect against *S. rolfsii*.

The aim of the present study was to identify the nature of the inhibitory compound, which was produced as a result of adding ammonium acetate in the microbial medium of *B. psychrosaccharolyticus*, against *S. rolfsii*. Furthermore, studding

effect of this inhibitor on inhibition, killing of fungal growth and killing of sclerotia of other plant pathogenic fungi.

MATERIALS AND METHODS

Materials:

Ammonium acetate, sodium acetate, ammonium dihydrogen phosphate, ammonium sulfate, ammonium oxalate and ammonium citrate 89-99% were obtained from Adwic Co.

The plant pathogenic fungi *Sclerotium rolfsii*, *Sclerotium cepivorum*, *Sclerotinia sclerotiorum*, *Fusarium solani*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Macrophomina sp.* were obtained from Plant Disease Department, Faculty of Agriculture, Ain Shams University.

Potato Dextrose Agar medium (PDA) was obtained from Biolife Co. This medium was used for maintenance of all pathogenic fungi and in the inhibition test.

Methods:

1- Inhibition effect of ammonium acetate: a solution of 1.3% ammonium acetate (pH was adjusted to 5.6 by acetic acid), 1.0% glucose and a mixture solution (1.3% ammonium acetate + 1 % glucose, pH 5.6) were sterilized in autoclave for 15 min. at 121˚C. These concentrations of ammonium acetate and glucose were used also in the microbial medium of *Bacillus psychrosaccharolyticus*. Five ml of each of these solutions was mixed with five ml of PDA medium (8% instead of 4%) and mixed well in 5-cm diameter Petri dish. The media after solidification were inoculated with a 1-cm diameter disk of any of the seven pathogenic fungi. After incubation at 28˚C for 7-days, the growth diameter was measured and compared with the control growth. Three replicates were made from each treatment.

2- Inhibition effect of acetate ion: Different amounts (0.05g, 0.1g, 0.25g and 0.5g) of either Ammonium acetate, sodium acetate, ammonium dihydrogen phosphate and ammonium sulfate was dissolved in 100 ml of distilled water and pH was adjusted to 5.6 by the conjugate acid (acetic acid for acetate salts, Phosphoric acid for phosphate salt and sulfuric acid for sulfate salt). To each solution 4 g of PDA was added and the media were then sterilized in autoclave for 15min. at 121 ˚C. The media were poured in 5-cm diameter Petri dishes. After solidification the media were inoculated by a disk of

Sclerotium rolfsii, Sclerotium cepivorum or *Sclerotinia sclerotiorum*. Three replicates were made from each treatment. The dishes were incubated at 28˚C for five days. The growth diameter of each fungus was measured at the end of incubation period and compared with the control growth.

3- Killing effect of acetate ion: Different amounts (1g, 2.5g and 5g) of ammonium acetate were dissolved in 100ml of distilled water and the pH was adjusted to 5.6 by acetic acid. Ten ml of each of these solutions was added in Petri dishes in which the plant pathogenic fungi (*Sclerotium rolfsii, Sclerotium cepivorum* or *Sclerotinia sclerotiorum*) were fully grown on PDA medium. The control was made from each fungus by treating with ten ml of sterilized distilled water instead of the salt solution. After one and two hours of soaking, one-cm diameter disk of each treated fungi was taken and cultured on PDA medium. Three replicates were made from each treatment. The dishes were incubated at 28˚C for five days. The growth diameter of each fungus was measured at the end of incubation period and compared with the control growth.

4- Killing of sclerotia: Three sclerotia of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* and several sclerotia of *Sclerotium cepivorum* (about 0.1g) were surface sterilized by 70% ethanol for five minutes. The sclerotia were then soaked in different concentrations of ammonium acetate solution (1%, 2.5%, 5% and 10%, pH 5.6) for 1, 2 and 24 hours. The control was made by soaking the sclerotia in sterilized distilled water for the same periods. The sclerotia were then washed by sterilized distilled water and cultured on PDA. Three replicates were made from each treatment. The dishes were incubated at 28˚C for seven days. The growth was compared with the control.

5- Effect of other carboxylic acids: PDA media containing different concentrations (0.05%, 0.1%, 0.25 and 0.5%) of either ammonium oxalate or ammonium citrate (pH 5.6) was inoculated with each of S. *rolfsii, S. cepivorum* and *S. sclerotiorum*. Three replicates were made from each treatment. The dishes were incubated at 28˚C for five days. The growth was compared with the media containing the same concentrations of ammonium acetate and control.

RESULTS AND DISCUSSION

1- Inhibition effect of ammonium acetate:

Firstly we suggested that the inhibition effect may be caused by the pyrolysis products of ammonium acetate and glucose during sterilization in the autoclave which may be formed as described by Daisuke and Hitoshi 1982. They reported that mutagenic compounds are formed when ammonium salts alone or with glucose are heated at high temperature. These mutagenic compounds are described as pyrolysis products. Therefore we tested the autoclaved ammonium acetate solution, the glucose solution and the mixture of ammonium acetate and glucose solution for its inhibition effect against the seven plant pathogenic fungi. We found that both ammonium acetate and the mixture solutions showed inhibition effect against three fungi *S. rolfsii, S. cepivorum* and *S. Sclerotiorum*. Therefore we suggested that the ammonium acetate was the compound that has the inhibitory effect. To decide whether the ammonium acetate salt itself or its pyrolysis products that have the inhibitory effect, the inhibition test was carried out by using both autoclaved ammonium acetate solution and the solution of ammonium acetate dissolved in sterilized water (not autoclaved solution). The inhibition effect for the three fungi was obtained by both solutions. This result indicated that the ammonium acetate salt itself not its pyrolysis products (if there) has an inhibitory effect against *S. rolfsii, S. cepivorum* and *S. Sclerotiorum* (Table 1).

Table (1): Effect of sterilized and non- sterilized ammonium acetate, sterilized glucose and mixture solutions on seven plant pathogenic fungi.

Tested solution / Tested fungi	CH_3COONH_4 (non sterilized)	CH_3COONH_4 (sterilized)	CH_3COONH_4 + glucose	Glucose solution
S. rolfsii	100%	100%	100%	-
S. cepivorum	100%	100%	100%	-
S. sclerotiorum	100%	100%	100%	-
F. Solani	-	-	-	-
F. oxysporum	-	-	-	-
Macrophomina sp.	-	-	-	-
Rhizoctonia solani	-	-	-	-

2- Inhibition effect of acetate ion: To decide whether the ammonium ion or acetate ion has the inhibitory effect, different concentrations of two acetate salts (ammonium acetate and sodium acetate) and two ammonium salts (ammonium dihydrogen phosphate and ammonium sulfate) were used in inhibition test against the three fungi. Both ammonium acetate and sodium acetate showed inhibition of the three pathogenic fungi. *S. rolfsii* was completely inhibited at 0.1% concentration of both sodium and ammonium acetate solution; while *S. cepivorum* and *S. Sclerotiorum* were completely inhibited at higher concentration (0.2%) of both acetate salts also (Table 2). Neither ammonium dihydrogen phosphate nor ammonium sulfate salts showed inhibition effect against any fungi. These results indicated that the acetate ion itself was the inhibitor of these three fungi. In addition to that, *S. rolfsii* was more susceptible to acetate ion than the other two fungi (*S. cepivorum* and *S. Sclerotiorum*). These results were in agreement with Erman and Miko (2005). They found that acetate ion inhibits the growth of several wood rotting fungi including brown-rot and white-rot fungi out of 24 tested fungi. They also suggested that: A possible mechanism involved in inhibition of fungal growth caused by acetate is explained by suppression of glucose absorption probably by reducing or inhibiting the normal function of glucose receptor in the cell membrane, but this mechanism requires more studies for confirmation.

3- Killing effect of acetate ion: Treatment of grown fungi by soaking in several concentrations of ammonium acetate for different periods showed that: *S. rolfsii* was killed after treatment by 2.5% of ammonium acetate solution (pH 5.6) for only one hour; while both *S. cepivorum* and *S. Sclerotiorum* were killed after treatment with higher concentration (5%) for also one hour. This result confirmed the susceptibility of *S. rolfsii* to acetate ion than the other two fungi (*S. cepivorum* and *S. Sclerotiorum*) as shown from the previous experiment.

4- Killing of sclerotia by acetate ion: Effect of acetate ion concentration on viability of sclerotia showed that the sclerotia of both *S. rolfsii and S. cepivorum* were killed after treatment by 5% of ammonium acetate solution (pH 5.6) for one hour only; while the sclerotia of *S. sclerotiorum* showed resistance to this method, although

using high concentrations of ammonium acetate up to 10% and long time of treatment up to 24 hours.

5- Effect of other carboxylic ions: Ammonium oxalate and ammonium citrate showed no inhibition effect against any of the three fungi.

Table (2): Inhibition effect of different concentrations of two acetate salts and ammonium salts on the three plant pathogenic fungi.

Salt	Salt concentration	Inhibition %		
		S. rolfsii	*S. cepivorum*	*S. sclerotiorum*
CH_3COONH_4	0.05%	95%	0.0%	0.0%
	0.1%	100%	80%	70%
	0.2%	100%	100%	100%
	0.4%	100%	100%	100%
CH_3COONa	0.05%	95%	0.0%	0.0%
	0.1%	100%	80%	70%
	0.2%	100%	100%	100%
	0.4%	100%	100%	100%
$NH_4H_2PO_4$	0.05%	0.0%	0.0%	0.0%
	0.1%	0.0%	0.0%	0.0%
	0.2%	0.0%	0.0%	0.0%
	0.4%	0.0%	0.0%	0.0%
$(NH_4)_2SO_4$	0.05%	0.0%	0.0%	0.0%
	0.1%	0.0%	0.0%	0.0%
	0.2%	0.0%	0.0%	0.0%
	0.4%	0.0%	0.0%	0.0%

CONCLUSION

Presence of 0.1% of ammonium acetate, pH 5.6 (corresponding to 0.015 molar of acetate ion) in the growing media of *S. rolfsii* prevented its growth. While 0.2% of ammonium acetate, pH 5.6 (corresponding to 0.03 molar of acetate ion) prevented the growth of *S. cepivorum* and *S. Sclerotiorum*. The grown *S. rolfsii* can be killed by spraying with 2.5% of ammonium acetate, pH 5.6 (corresponding to 0.36 molar acetate ion). While *S. cepivorum* and *S. Sclerotiorum* can be killed by spraying with 5% of ammonium acetate, pH 5.6 (corresponding to 0.74 molar of acetate ion). The sclerotia of both *S.*

rolfsii and *S. cepivorum* can be killed by treatment with 5% of ammonium acetate, pH 5.6 (corresponding to 0.74 molar of acetate ion); while Sclerotia of *S. sclerotiorum* were resistant.

Pots and field experiments will be designed to study the effect of ammonium acetate on the three plant pathogenic fungi *S. rolfsii*, *S. cepivorum* and *S. Sclerotiorum* in presence of its target plants.

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أيون الأسيتات مثبط لبعض فطريات التربة الممرضة للنبات

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وجد أن ايون الأسيتات لـه أثر مثبط لثلاثة من فطريات التربة الممرضة للنبات و التى تكون أجسام حجرية و هى:

Sclerotium rolfsii, Sclerotium cepivorum and *Sclerotinia sclerotiorum.*

و لم يظهر أثر مثبط لأربعة فطريات أخرى ممرضة للنبات و هى:

Fusarium solani, Rhizoctonia solani, Fusarium oxysporum and *Macrophomina sp.*

تم تثبيط نمو فطر *S. rolfsii* عند تركيز أيون الأسيتات ٠,٠١٥ مولر، و نمو فطر *S. Sclerotiorum* و *S. cepivorum* عند تركيز ٠,٠٣ مولر فى البيئة. تم قتل الفطر النامى بعد المعاملة لمدة ساعة واحدة بتركيز أيون الأسيتات ٠,٣٦ مولر و ذلك لفطر *S. rolfsii* و تركيز ٠,٧٤ مولر لكلا من فطر *S. Sclerotiorum* و *S. cepivorum* .

تم قتل الأجسام الحجرية لكلا من فطر *S. rolfsii* و *S. cepivorum* بعد المعاملة بتركيز أيون الأسيتات ٠,٧٤ مولر لمدة ساعة واحدة. الأجسام الحجرية لفطر *S. Sclerotiorum* لم نتمكن من قتلها على الرغم من استخدام تركيز عالى من الأسيتات حتى ١,٥ مولر و لمدة زمنية طويلة وصلت الى ٢٤ ساعة. تم اختبار املاح كربوكسيلية أخرى (أوكسالات الأمونيوم و سترات الأمونيوم) و لكنها لم تظهر أى تثبيط ضد أى من الفطريات الثلاثة.