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Study on Antifungal Activity of Some Salts on Growth and Dry Rot Development of *Fusariumsolani* (Mart.) Sacc.

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Abstract: PotatoFusariun dry rot is one ofthe most important diseases of potato in the world. The disease cause significant losses in both quality and quantity of seed tubers. For many years, Chemical fungicideswere used against *Fusarium* spp. In this study, antifungal activity of some salts (ammonium phosphate, Potassium carbonate, Potassium bicarbonate, sodium carbonate and sodium bicarbonate) was investigated against *Fusariumsolani*. *In vitro*, Inhibition of the growth rate was evaluated after 7 days in PDA media. The results exhibited the salts had inhibitory activities on the growth rate of this fungus.Ammonium phosphate indicated the largest inhibition (92.29%) on the growth rate of *F. solani* in PDA media and followed by potassium carbonate (54.92%), potassium bicarbonate (46.14%), sodium carbonate (42.60%) and sodium bicarbonate (42.33%). *In vivo*, the results indicated ammonium phosphate had maximum inhibition (0.5 cm^2) of potato dry rot development after 2 month in storage condition.Our study provedammonium phosphate was the best treatment under both *in vitro* and *vivo*.

Key words: Antifungal activity · *Fusariumsolani* · Salt · Inhibition

INTRODUCTION

Potato (*Solanumtuberosum* L.)is very susceptible to infection by various diseases [1]. Fusarium dry rot of potatoes is one of the most economic problems in the world [2] and cause greater losses of yield than any other post-harvest disease [3]. The diseases of potato lead to significant losses in both quality and quantity of seed [4.*Fusarium* spp.kill potato sprouts and reduce crop establishment and the crop losses can occurred up to 25 percent. These pathogens can be infected more than 60 percent of tubers in storage [5].

Management of *Fusarium*spp. is very difficult because these fungi survive for long time as mycelium in organic matter under different conditions [6]. Wharton and Kirk [5] reported the most potato cultivars are susceptible to dry rot, but the cultivars reaction was different. Their results demonstrated several potato lines have a higher level of resistance to dry rot.

Chemical fungicides traditionally have been used against plant pathogenic fungi. Frequency usage of the synthetic fungicides leaded to the development resistant population of the fungi, increase production costs, and environmental and human health hazards [7-8].

Thiabendazole is one of the fungicidesused as post-harvest treatment for controlling dry rot but there are different reports about resistance of the pathogen against the fungicide [9-11]. The fungicide leaded to increasing of dry rot incidence and severity [12].

Although different cultural practices can be used to decrease dry rot [12], alternative control strategies are necessary. Some organic and inorganic salts with antimicrobial properties are widely used in the food industry as preservatives and antimicrobial agents to control of plant pathogenic fungi [13]. Olivier *et al*. [14] proved, the compounds revealedbroad-spectrum antimicrobial properties with low mammalian toxicity.

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Ziv and Zitter [15] studied effect of several salts against plant pathogenic fungi and the results showed spraying of bicarbonate salts, provided good control of different disease in greenhouse.Punja and Gaye [16] showed post-harvest application of different salts reduced black root rot of carrots.

Mecteau *et al*. [17] investigated effect of different salts against *F. solani* var. coeruleum. The results stated aluminium acetate, aluminiumchloride, sodium benzoate, sodium meta bisulfate, potassium sorbate and trisodium phosphate completely inhibited mycelial growth. The effect of different salts against conidia mortality exhibited aluminium acetate, potassium sorbate, sodiumbenzoate, sodium metabisulfite or trisodium phosphate at 0.2 M leaded to 100% mortality of theconidia after 1 h while aluminium chloride and aluminium lactate showed 100% mortality after anexposure of 24 h. Study on efficacy of salts on potato dry rot development demonstrated only aluminum chloride exhibited a significantreduction in potato dry rot compared with the control.

The objectives of this study were to determine the effect of ammonium phosphate, Potassium carbonate, Potassium bicarbonate, sodium carbonate and sodium bicarbonate on the growth rate and dry rot severity caused by *F. solani* in potato tubers.

MATERIAL AND METHODS

Fungal Isolation: Infected Tubers were collected from different regions of Mojen, shahroud, Iran. The infected tissues were sterilized in 96% ethanol for 1 min, and washed with sterilized water. The small pieces of the tissues were excised and placed on the Petri dishes containing PDA and incubated at 25°C. Purification was carried out using single spore method and fungi associated with dry rot tubers were isolated and identified according to document [18-19].

The purified fungi were grown on PDA medium and incubated at 25°C for8 days and used for preparing spore suspensions. Spore suspensions were adjusted to 5x 10^5 spore/ml for inoculating potato tuber.

Effect of different salts on mycelia growth of *F. solani*: The efficacy of different salts and concentrationswas studied on the mycelial growthof *F.solani*. The evaluated salts were ammonium phosphate, Potassium carbonate, Potassium bicarbonate, sodium carbonate and sodium bicarbonate. PDA disk (6 mm diam.) of *F. solani* were grown on PDA unamended (control) and amended with the salts at 3, 6 and 9 ppm. at 24±2°Cfor 7 days. The experimental design was a completely randomized block with three replicates. Mean diameter of colony was measured and inhibition of mycelial growth was calculated as Pandey *et al*. [20] formula:

[(control radial growth - salt-amended radialgrowth) / control radial growth] x 100.

Effect of Salts on Potato Dry Rot Development: In order to evaluation effect of ammonium phosphate, potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate on potato dry rot development, tubers were washed according to reference documents [17]. Four wounds (4 mm deep) were carried out on each tuber by a cork borer.Tubers were inoculated with conidia (5 x 10^5 conidia mL^{-1}) of *F. solani* obtained from 8 days old culture grown on PDA. The suspensions were injected into each wound and inoculated tubers incubated in the dark at 24°C for 24 h. Then, tubers were dipped (10 min) in the different salt solutions (1, 2 and3 g/L) or in distilled water (control) and incubated individually in the dark at 24°C in plastic chambers containing a moistened towel for 2 month. The experimental design was a completely randomized block with three replicates. Disease severity evaluated by lesion area (cm^2) [21].

RESULTS

In order to investigate the effect of inorganic salts on growth inhibition isolate *F. sola*ni, diameter of fungal colonies on PDA medium containing mineral salts of different treatments after 7 days was measured.Data were analyzed were performed using MS-Excel and MSTAT-C program.

Different concentrations (3, 6 and 9ppm) of the salts from fivesalts were tested against *F. solani* to determine their antifungal activity *in vitro* tests. The results of analysis of variance revealed that all tested salts caused significant inhibition of mycelia growth of the fungi (Table 1). Furthermore, data analysis showed the differences between salts are significant ($p<0.01$).

Means comparison test based on Duncan at the 95% probability level showed that there was differences in the growth inhibitory effects of various treatments and treatments were placed in different groups.Ammonium phosphate with an average of 99.29% inhibition of growth

Table 1: ANOVA table for the effect of different concentrations of some salts in inhibition of *Fusariumsolani* mycelia growth.

Source of variances	Degree of freedom	Sum of square	Means of square	F test
Salts	2	16030.228	4007.557	30.9605**
Concentration	4	321.655	160.828	1.24 ns
Salts ×Concentration	8	1215.039	151.880	1.17ns
Error	30	3883.223	129.441	
Total	44	21450.145		
CV	20.44%			

P value<0.01

Table 2: Inhibition percent of mycelia growth of *F. solani* by different concentrations of salts on PDA.

	Concentrations (ppm)		
SALTS	3	6	9
Ammonium phosphate	76.87AB	100A	100A
potassium carbonate	55.92C	52.90C	55.93C
potassium bicarbonate	38.08C	41.14C	47.76C
sodium carbonate	38.42C	41.34C	48.05C
sodium bicarbonate	51.26C	46.09C	41.08C

Means within each column having the same letters are not significantly Different

Table 3: ANOVA table for the effect of different concentrations of some salts in inhibition of *Fusariumsolani*on potato dry rot development

Source of variances	Degree of freedom	Sum of square	Means of square	F test
Replication	2	0.010	0.005	0.20ns
Salts	5	9.925	1.985	81.65**
Concentration	2	0.188	0.094	3.86ns
Salts ×Concentration	10	0.276	0.028	1.13ns
Error	34	0.827	0.024	
Total	53	11.225		
CV	10.37%			

P value<0.01

rate, showed maximum inhibition and placed in group A, and followed by potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate salts(group B)with 54.92, 46.14, 42.60 and 42.33% inhibition respectively.

Theinvestigation of different salt concentrations on colony growth showed thatthere wasno statistically significant difference among of the concentrations of 3, 6 and 9 ppm.with a mean growth inhibition 58.57, 56.29 and 52.11% respectively.

study on the interaction between salts and concentrations on the inhibition of colony provedthere was no significant difference but the mean comparison analysis of interaction between salts and concentration exhibited ammonium phosphate(6 and 9 ppm) with a mean of 100% inhibition of growth rate of *F. solani* had the greatest effect and placed instatistical group A. Ammonium phosphate salt of 3 ppm with 87.76% inhibition of growth rate of the fungus located after the treatments listed (AB group) and other treatments were in group C.In this study, the least inhibition of growth rate was observed at 3 ppm.potassium bicarbonate salt (Table 2).

The Effect of Different Concentrations of Mineral Salts on Potato Dry Rot Development: To study the protective effect of salts on disease control, measurement of disease lesion area on the inoculated tuber was performed after 2 months instore.Data were analyzed using MSTAT-C software.

Different concentration (1, 2 and 3 g/l) of the salts were evaluated against *F. solani* to determine their antifungal activity in *vivo*. The results of analysis of variance showed that all tested salts caused significant inhibition of dry rot development (Table 3). Furthermore, data analysis exhibited the differences between salts are significant ($p<0.01$).

Means comparison test based on LSD at the 95% probability level showed that there was differences in the inhibitory effects of different treatments, and the treatments were placed in different groupsstatistically.

In order to evaluate the effect of salts on potato dry rot development, inoculated tubers with *F. solani*were treated with the different salts and disease severity was evaluated following an incubation period of 2 month. Among the test saltsAmmonium phosphate with an average lesion area of 0.05 cm^2 was the most effective in inhibiting the dry rot developmentand Placed in group D, and potassium carbonate and potassium bicarbonate, with an average lesion area of 1.31 and 1.40 respectively, placed in group C.Sodium carbonate and sodium bicarbonate salts, with an average lesion area of 2.55 and 2.44 cm^2 respectively, placed in group B and control treatment with an average lesion area of 4 cm^2locatedin group A.

Inhibition effect of different salt concentrations (1, 2 and 3 g/l) on the fungal development on the tuber (with an average lesion area of1.90, 1.84and1.52 respectively) showed there was no statistically significant among of treatmentsandallwereinthe same statistical group.

There was no significantdifference on study of the interaction between the salts and concentration in the inhibitory of dry rot development on tuber but the grouping was determined that potato tubers, as well as were completely protected against infection by ammonium phosphate salt (2 and 3g/l) and followed byammonium phosphate 1 g/l, with a lesion area of 0.15 cm^2 (GF).

Table 4: Effect of salts on the development of potato dry rot caused by Fusariumsolani($cm2^2$)

	Concentrations (g/l)		
SALTS	1	2	3
Ammonium phosphate	0.15FG	0G	0G
Potassium carbonate	1.63CD	1.46CDE	0.65EF
Potassium bicarbonate	1.81CD	1.46CDE	1.14DE
Sodium carbonate	2.48BC	2.45BC	2.48BC
Sodium bicarbonate	1.72BC	2.98AB	2.12BCD
Control (without salt)	4.1A	4.1A	3.8A

Means within each column having the same letters are not significantly Different (LSD 5%).

In this study, the least inhibition in control treatment with lesion area 4 cm^2 and followed by sodium bicarbonate 2, sodium carbonate 3, sodium carbonate 1, Sodium bicarbonate 1 and Sodium carbonate 2 g/l, , with lesion area of 2.98, 2.48, 48/2, 2.48and 2.45 cm^2respectively (Table 4).

DISCUSSION

Dry rot is an important potato tuber disease caused by different species of *Fusarium*, including *F.solani* var. *coeruleum* and *F. sambucinum*. With the appearance of resistant strains of F. sambucinum to thiabendazole, a fungicide that made it possible to control potato dry rot, increased incidence and severity of the disease were observed. In an attempt to develop alternative strategies for the control of potato dry rot, Mecteau *et al*. [17] tested several organic and inorganic salts for their effect on dry rot caused by *F. sambucinum*. They showed that specific salts, when applied on potato tubers, made it possible to reduce disease severity, thus suggesting that salts may eventually be used in the control of potato dry rot. The present study showed that F. solani development is strongly affected by several salts. In fact, the mycelial growth of the fungus was completely inhibited by ammonium phosphate, potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate salts. These results are in agreement with those of Mills *et al*. [22]. Theirreport proved that these salts inhibited F. solanimycelial growth. Among the test salts, ammonium phosphate was also shown to completely inhibit mycelial growth. Moreover, the results showed that exposure ammonium phosphate, potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate salts was toxic to *F. solani*. The toxicity of these salts against *F. sambucinum* conidia had previously been observed by Mecteau *et al*. [17].

Ammonium phosphate (at concentrations of 6, 9 ppm) were found to be more effective when compared to potassium and sodium salts *in-vitro* condition, andthen ammonium phosphate 100, potassium and sodium saltswere identifiedas themost effective. In the present study it is evident that mineral salts suppress tuber rot on *potato*, and were found effective. It is observed that inhibition of both mycelium growth and tuber dry rot even at lower concentrations *invitro*.

Several reports exist on the effect of inorganic salts on disease control. Zivand Ziter [15] evaluatedeffect offoliar application of sodium bicarbonate in control water melonearly blight diseasecaused by *Alternariacucumerina* reported in the greenhouse. Horst *et al*. [23] showed that powdery mildew disease caused by *Spherothecapannosa* var. *rosea* and Rose black spot caused by *Diplocarponrosea* can be controlled with the use of sodium bicarbonate solution containing 1% oil sun spray.

According to Punja and Gaye [16], using solutions of ammonium bicarbonate, sodium bicarbonate, sodium carbonate and potassium sorbate on the control of black root rot of carrots caused by *Chalaraelegans* are effective.

Olivier *et al*. [14] reportedthat some salts such asammonium bicarbonate, sodium carbonate, sodium bicarbonate, sorbate, potassiumcarbonateandpotassium bicarbonate Helminto sporium potato spotdiseasecaused by *H.solani* substantially reduce. They showed that these salts notonly broad-spectrum antimicrobial, but also toxicity to mammals are slow.

Although Sodium and Potassium salts were practiced to control many fungal diseases, no significant work has been done to control post-harvestFusarium dry rotand also the chemicals selected were commonly used as additives or food preservatives and are considered as safe to the environment and human health when treated with suitable doses. From the results it is made clear that both sodium and potassium salts have ability to control *F.solani*both *invitro* and *invivo.*

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