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EFFECT OF SALICYLIC ACID SOLUTION ON FUNGUS *RHIZOCTONIA SOLANI* AND SEED GERMINATION

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Abstract

The main aim of this search is to determine the effect of salicylic acid solution at different concentration against pathogen *Rhizoctonia solani*, which caused wilt disease and seed decay. The result showed a significant positive relation between concentrations of salicylic acid and the mean percentage inhibition of fungus *R. solani* in Petri dish and presoak of cucumber seed for period within 24 hours promoted increased germination by high percentage emergence. While , presoak of cucumber seed for period more than 24 hours have negative double effect by inhibition pathogen growth and cause seed dormancy.

Key words : Salicylic acid, soil born fungi, antifungal activity, seed germination.

Introduction

Rhizoctonia solani (Kühn) [teleomorph *Thanatephorus cucumeris* (Donk)] is a necrotrophic soil-borne fungus belonging to the phylum Basidiomycota (Anderson, 1982), that caused wilt disease and seed decay (Agrios, 2005; Drizou *et al*., 2017; Dina and Ahmed, 2016). Numerous approaches were used to control these disease (Tariq *et al*., 2010; Ahmed *et al*., 2016) achieved by different fungicides (Boutin *et al*., 2014), soil fumigants and bio agents (Boutin *et al*., 2014; Deising *et al*., 2008). Regrettably, most these approaches have unpleasant effect due to the development resistant strains of pathogens against various chemical fungicides (Deising *et al*., 2008; Zafra *et al*., 2015). Salicylic acid is monohydroxybenzoic acid (Alam *et al*., 2013; Hayat *et al*., 2010), that biosynthetic via 2 pathways: Phenylalanine ammonia lyase (PAL) and Isochorismate synthase (ICS) (Arberg, 1981). It is a natural ,phenolic compound from White willow (*Salix alba*), that affects a variety of biochemical and molecular events associated with induction of disease resistance (Chandra and Bhatt, 1998; Eraslan *et al*., 2010; Gharib, 2006) the phenolic compounds present as fungitoxic, antibacterial and anti-virotical activities (Gharib, 2006; Mandal *et al*., 2009). Therefore, the present investigation was to evaluated use salicylic acid solution as natural compound against soil-borne fungus *R. solani in vitro*.

Materials and Methods

Pathogenic fungi

Soil-borne fungus *Rhizoctonia solani*, which was isolated from infection soil . After series purification , the fungus transport to culture of potato dextrose agar (PDA) media and identify depended on Anderson (1982) and Tsuneo (2002) on potato dextrose agar (PDA).

Antifungal activity assay by agar diffusion

The anti–fungus activity was preceded accorded to Xia *et al.* (2012) by mix different concentrations of salicylic acid solution concentration (0.5, 1, 2, 4 and 8 mg/ml) and incorporated into PDA medium just before pouring in sterilized Petri dishes. A plug (2.5 cm diameter) of mycelia and spores taken from the periphery of 10-day-old culture *R. Solani* that incubated at 28±2°C for 7 days. The experiment has run in six replicate.

The radial growth of the colony was measured. Inhibition% of mycelia growth was calculated as fallowing:

$$\text{Inhibition\%} = [(R1 - R2)/R1] \times 100$$

Where, R1 = the Radius of normal growth in control plates, R2 = the Radius of inhibited growth.

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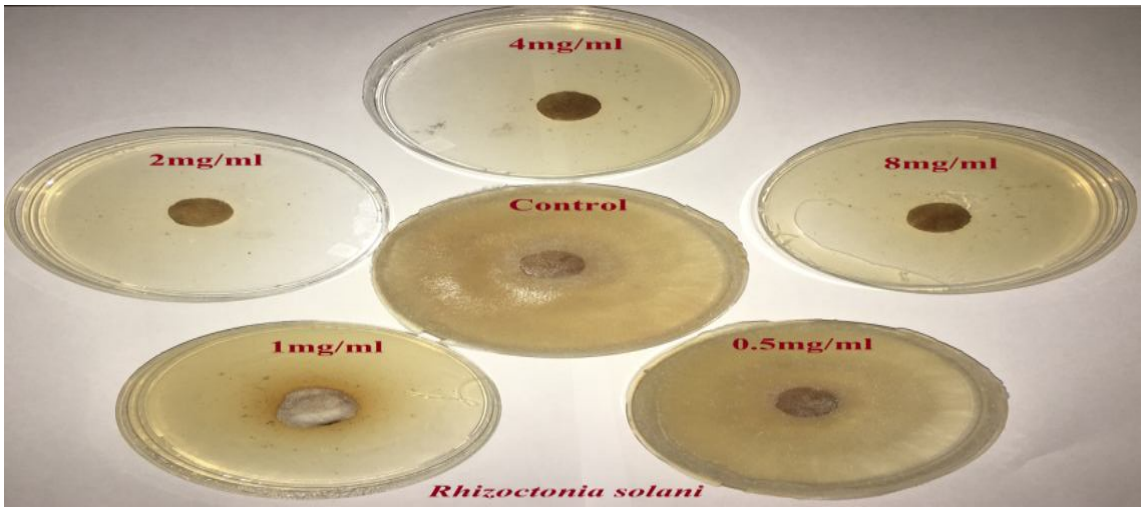

Fig. 1 : Growth inhibition of *R. solani* in PDA plates at different concentration of salicylic acid for seven days.

Table 1 : Inhibin percentage of the fungus *R.solani* by using salicylic acid at different concentration (mg/ml) on PDA plates through 7 days at 2 ±28^{0}C.

Concentration (mg/ml)	Time (hr.)							L.S.D 0.05
	24	48	72	96	120	144	168	
Control	72.22*	64.44	54.44	31.11	13.33	5.55	0.00**	3.45*
0.5	72.22	66.66	58.88	35.55	18.88	0.00	0.00	7.36*
1	72.22	66.66	66.66	66.66	66.66	66.66	66.66	0.00*
2	72.22	72.22	72.22	72.22	72.22	72.22	72.22	0.00*
4	72.22	72.22	72.22	72.22	72.22	72.22	72.22	0.00*
8	72.22	72.22	72.22	72.22	72.22	72.22	72.22	0.00*
L.S.D 0.05	0.00NS	0.01NS	0.2NS	0.3NS	0.00NS	0.07NS	0.03NS	

* (P<0.05), NS: Non-Significant. *Disc dimeter of R.solani inoculum was 2.5cm. ** Dimeter of plat was 9 cm

Pre-soaking seeds in different concentrations of salicylic acid

In this experiment, seeds of *Cucumis sativus* L. (from local mark) were surface sterilized as previously described and presoaked in the different concentrations of salicylic acid solution at concentration (0.5, 1, 2, 4 and 8 mg/ml) for 12, 24, 36 and 48 hr., the control seeds were presoaked in sterile distilled water for 24 hr. At the end of the presoaking period, ten seeds of each treatment planted on each Petri dish agar media. The petri dishes were inoculated each alone at the center with 9 mm inoculum-disc of each test fungus *R. solani*, then incubated at 28±2°C for 14 days. They compared with positive control (seed without fungal inoculums) and negative control (seed with fungal inoculums). Six replications were maintained for each treatment (Rajjou *et al*., 2006).

Results and Discussion

The results reported in the current study showed a significant positive relation between concentrations of salicylic acid and the mean percentage inhibition of fungus *R. solani* however when the concentration of salicylic acid was high the percentage of fungus inhibition is high (table 1 and fig. 1). Data presented in table 1 that the percentage inhibition of anti-*R. solani* activity was varied between (72.22-0.00%). The lowest value was (0.00%) in day six and seven at 0.5 mg/ml concentration , while the highest value was (72.22)% along the week at 2,4 and 8 mg/ml.

Interestingly, it was found that SA was able to significantly reduce *R. solani* mycelia growth at 2,4 and 8 mg/ml concentration completely ,because SA is a natural phenolic compound contain monohydroxybenzoic acid with orthor and para position of OH- group (Cherif *et al*., 2007; Huang *et al*., 2009) that have inhibitory effect on microbial and that the reason to toxic effect on fungus (Abad *et al*., 2007; Ansari *et al*., 2013), while the effect of 0.5 and 1 mg/ml were less (limited) effective for reducing phytopathoginc fungus, that make microscopic changes in the mycelium, maybe due to low concentration of salicylic acid make fungus utilize tiny amount of SA

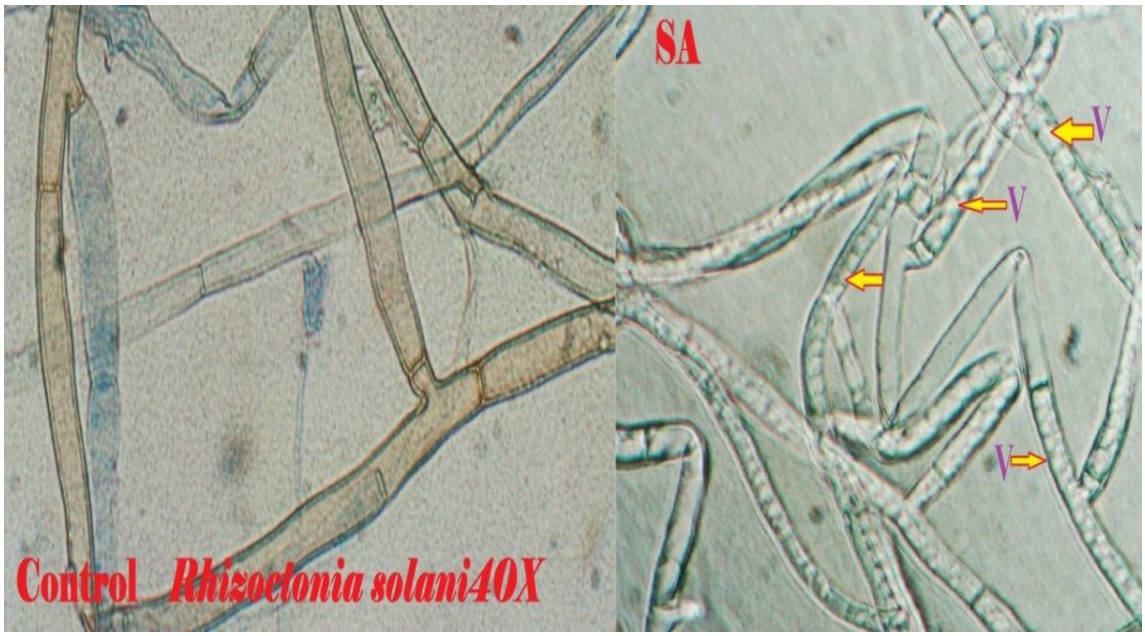

Fig. 2 : *R. solani* treatment with salicylic acid at 1mg/ml concentration. Noted the vacuoles and thinly hyphae.

and storage in vacuole (fig. 2). The vacuole is believed to be involved in fungus defense due to accumulates a variety of hydrolytic enzymes , like lysosomes and stored foreign molecular as a disposal site for waste and poisons for fungus defense strategies to protect fungal cell from death (Zafra *et al*., 2015; Richards *et al*., 2012).

Present vacuoles in fungus have numerous functions, including regulation of cellular functions such as degradation of intracellular components, growth and control of cellular morphology. Besides that, storage of metabolic products. Vacuoles are also highly dynamic, undergoing a continuous balance of fusion and fission reactions to allow changes in size, shape and number during cell division and turgor pressure (Richards *et al*., 2012; Veses *et al*., 2009; Richards *et al*., 2010). The results showed in table 2 and fig. 3 reveals that, the means of measures, which was taken in *C. sativus* seeds which soaked for 12, 24, 36 and 48hr. in Salicylic acid at 0.5, 1, 2, 4 and 8 mg/ml concentration and grew in a Petri dish contaminated with *R.solani*. That the mean of radical length ranged between 18.3 – 0.0 cm, which was the highest value (18.3) cm when soaked for 12 hr. at 8 mg/ml concentration.

The lowest value was 0.00 when soaked for 36 hr. at 8 mg/ml concentration and soaked for 48hr. at all concentration, compere with positive control that was 12.00cm. That the mean of plumule length ranged between (9.4 – 0.00 cm), which was the highest value (9.4) cm when soaked for 24 hr. at 2 mg/ml concentration. The lowest value was (0.00) when soaked for 36 hr. at 8 mg/ml concentration and soaked for 48 hr. at 2, 4 and 8 mg/ml concentration, compere with positive control that was 6.00cm. That the mean of No. Secondary Roots ranged between (27.2 -0.00 cm), which was the highest value (27.2 cm) when soaked for 24 hr.at 8 mg/ml concentration. The lowest value was (0.00) when soaked for 36 hr.at 8 mg/ml concentration and soaked for 48 hr. at 2, 4 and 8 mg/ml concentration, compere with positive control that was 20.0. That the mean of percentage ratio of seedling (%) ranged between (100-0.00%) cm which was the highest value (100%) when soaked for 12 hr. at 1, 2, 4 and 8 mg/ml concentration and soaked for 24 hr. at all concentration. The lowest value was (0.00) when soaked for 36 hr. at 8 mg/ml concentration and soaked for 48 hr. at all concentration, compere with positive control that was 100.

According to the results of this experiment, pre-soak seed with SA for period within 24 hr. show significantly affected promoted increased germination by high percentage emergence (Rajjou *et al*., 2006; Raskin, 1992) due to two reason, fistful it is a natural phenolic compound that plays a central role in certain physiological processes and defense responses in plants (Erashan *et al*., 2010; Misra and Misra, 2012; Anaya *et al*., 2017). Secondly, due to hydrolysis of complex into simple sugars that are readily utilized in the synthesis of auxins and proteins. Numerous studies have been performed to better understand the role of SA in induction or /and inhibition of dormancy seed (Abdel-Hai *et al*., 2009; Kabiri *et al*., 2014; Bentsinka and Koornneef, 2008).

When plant exposed to un convenient condition such as biotic or abiotic stress. Present SA at a small amount

Table 2 : *C. sativus* seeds soaked in Salicylic acid at different concentration and inoculums with *R. solani*.

Soak for 12 hr.								
Measures	**SA concentration (mg/ml)**					**Control**		**LSD**
	0.5	**1**	**2**	**4**	**8**	+	-	
Radical length (cm)	7.00	10.0	14.3	13.4	18.3	12.0	0.00	6.43*
Plumule length (cm)	4.2	6.00	7.8	11.6	12.8	6.0	0.00	3.89*
No. Secondary Roots	12.1	19.3	22.2	23.2	26.3	20.0	0.00	10.9*
Percentage ratio of seedling(%)	67.9	100	100	100	100	100	0.00	0.52*NS
LSD	2.2*	3.7*	5.1*	8.9*	9.35*	4.65*	0.00NS	
Soak for 24hr.								
Measures	**SA concentration (mg/ml)**					**Control**		**LSD**
	0.5	**1**	**2**	**4**	**8**	+	-	
Radical length (cm)	9	10.3	13.6	14.0	16.5	12.0	0.00	7.6*
Plumule length (cm)	5.1	7.6	9.4	8.1	12.3	6.0	0.00	3.3*
No. Secondary Roots	22.9	20.2	24.3	25.7	27.2	20	0.00	3.5*
Percentage ratio of seedling(%)	100	100	100	100	100	100	0.00	0.00NS
LSD	3.6*	4.9*	7.0*	7.3*	9.8*	5.7*	0.0NS	
Soak for 36hr.								
Measures	**SA concentration (mg/ml)**					**Control**		**LSD**
	0.5	**1**	**2**	**4**	**8**	+	-	
Radical length (cm)	8.4	4.5	2.4	2.1	0.00	12.0	0.00	1.2*
Plumule length (cm)	5.8	3.3	1.4	2.3	0.00	6.0	0.00	1.00*
No. Secondary Roots	6.3	3.7	4.2	5.2	0.00	20	0.00	2.4*
Percentage ratio of seedling(%)	9.0	9.4	7.8	6.1	0.00	100	0.00	4.3*
LSD	2.9*	2.6*	1.0*	1.8*	0.0NS	5.8*	0.00NS	
Soak for 48hr.								
Measures	**SA concentration (mg/ml)**					**Control**		**LSD**
	0.5	**1**	**2**	**4**	**8**	+	-	
Radical length (cm)	0.10	0.00	0.00	0.00	0.00	12.0	0.00	0.00NS
Plumule length (cm)	2.10	2.4	0.00	0.00	0.00	6.0	0.00	0.00NS
No. Secondary Roots	11.00	8.3	0.00	0.00	0.00	20	0.00	0.00NS
Percentage ratio of seedling(%)	7.00	4.00	0.20	0.00	0.00	100	0.00	0.00NS
LSD	0.00NS	0.00NS	0.00NS	0.00NS	0.00NS	0.00NS	0.00NS	

* ($P<0.05$).

can regulation of oxidative stress (Eraslan *et al*., 2010; Anaya *et al*., 2017; Radwan, 2012) because, it is a phenolic compound and endogenous phyto-hormones that plays interested role in growth and development of plant (Kabiri *et al*., 2014; Khan *et al*., 2012; Alam *et al*., 2013). Türkyilmaz *et al*. (2005) have found that salicylic acid promoted increased germination percentage in *Phaseolus vulgaris*, besides stimulating the length of roots and increasing green matter. In addition, the beneficial effect of salicylic acid they enhance the uptake of plant nutrients, impart a degree of frost resistance and make the plants better to resist the phytopathological fungi (Raskin, 1992; Misra and Misra, 2012).

Salicylic acid at high doses inhibited plant growth and

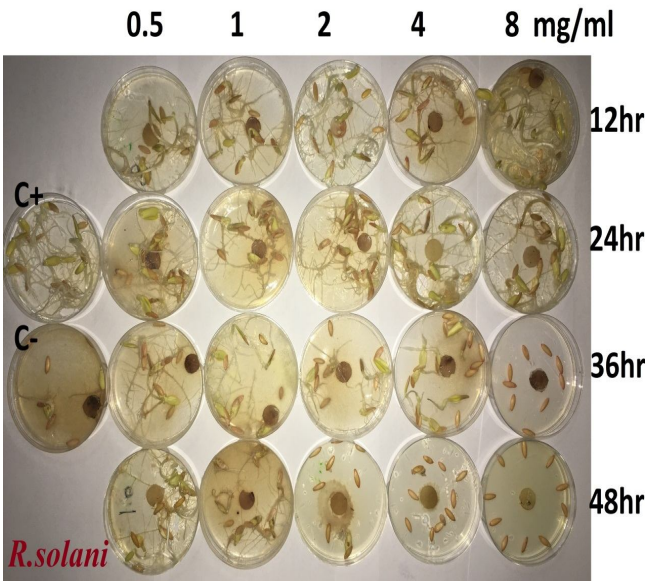

Fig. 3 : Pre-soaked of *C. sativus* in salicylic acid at different concentration for different period and inoculum with pathogen *R. solani*.

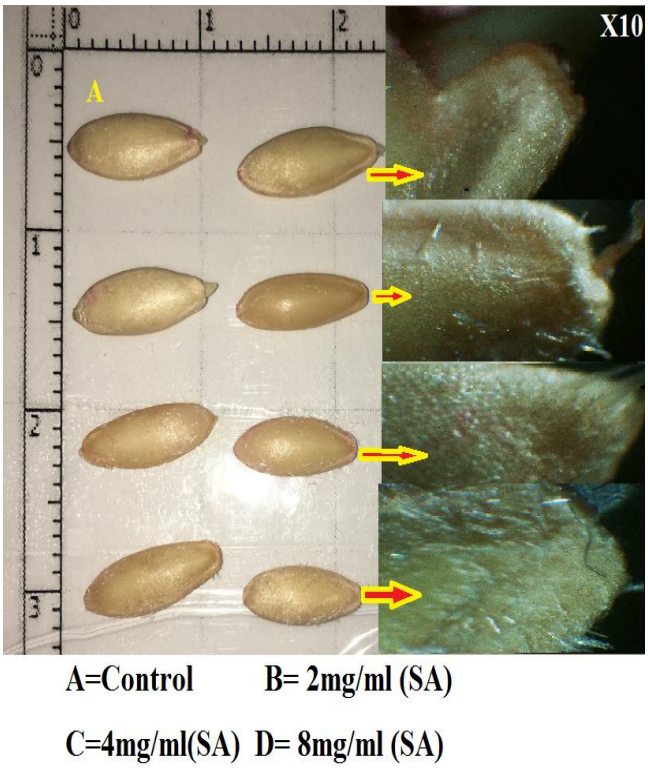

Fig. 4 : Surface of seed cucumber pre-soak in different concentration of Salicylic acid at magnification 10X.

chlorophyll contents in cucumber (Yildrim *et al*., 2008) and Tomato (Sabongari and Aliero, 2004). While, Tavares *et al*. (2014) observed no influence on seed quality of Rice seed which treatment with SA in concentration of 130 mg.L^{-1}.

SA treatment at 8mg/ml concentration for more than 24 hr. show binary effect by inhibition the pathogen

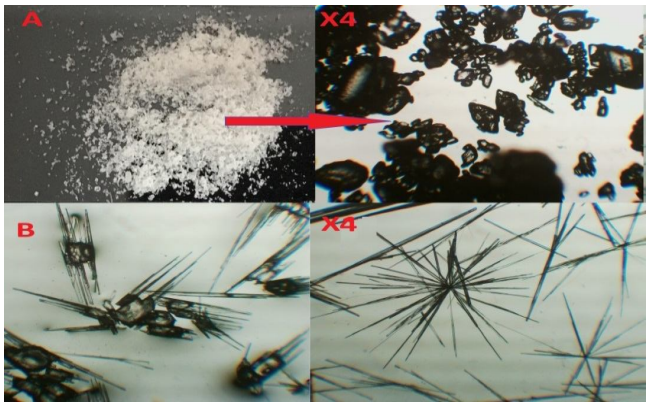

Fig. 5 : Salicylic acid (A) Powder of Salicylic acid (B) Dissolved Salicylic acid with ethanol 99%. Note that appear like needles.

development and caused seed dormancy. In another hand, SA treatment at the same concentration for less than 24 hr. promote increase seed germination and enhance shoot and root growth comparing with control treatment, because SA is a phenolic compound and endogenous phytohormone that plays an important role in plant growth and development (Sabongari and Aliero, 2004; Bentsinka and Koornneef, 2008; Farahbakhsh, 2012).

The most notable finding of this study was the optimal period of soaking seed within 24 hr. at 4 or/and 8 mg/ml concentration of SA to have positive double effect by inhibition pathogen growth and enhanced effects on seed germination and growth. While, pre-soaking seed more than 24 hr. at 4 or/and 8 mg/ml concentration of SA to have negative double effect by inhibition pathogen growth and cause seed dormancy. Salicylic acid induce and reduce at the same time some physiological processes and that depending on its concentration, species of plants, up growth stages and circumferential conditions (Rajjou *et al*., 2006; Sabongari and Aliero, 2004; Tavares *et al*., 2014; Jamshidi-Jam *et al*., 2012) and that explain variety effect of SA on seed germination (Rajasekaran *et al*., 2002). Seed soaking treatment with SA for short time enhanced germination in most seeds and prevent pathogen development, although, SA is not essential for germination under normal growth conditions. While, seed that soak for long time caused seed dormancy (Bentsinka and Koornneef, 2008; Sabongari and Aliero, 2004; Tavares *et al*., 2014; Farahbakhsh, 2012). SA is peel reagent (Arberg, 1981; Chandra and Bhatt, 1998), which caused scabrous-ness the surface of seed (figs. 4 & 5) make change on properties of seed coat and that lead to effect on testa and endosperm rupture which caused seed dormancy although, seed appear mature and swollen, but failure in radicle protrusion which prevent germination (Sabongari and Aliero, 2004; Tavares *et al*., 2014;

Jamshidi-Jam *et al*., 2012).

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