

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Efficiency of Salycilic Acid in the Reduction of *Cicer arietinum* Infection by *Fusarium roseum*.

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ABSTRACT

In Algeria, Fungal diseases are the most important biotic limiting the growth of Chickpea (*Cicer arietinum* L.). Salycilic acid application is known as a plant hormone that has the role of signal in responses of defense, whose the acquired systemic resistance. This study was aimed to evaluate the affectivity of some concentrations of Salicylic acid (SA) against the phytopathogenic fungus (*Fusarium roseum*) on two chickpea genotypes (ILC 3279 and FLIP 8555). Results showed that the inhibitory effect of (SA) on the development of *Fusarium roseum* increased linearly with increasing the concentration. The colony diameter reduced significantly at 200, 250 mg/ l. Additionally, our results showed that the different treatment of (SA) were effective in reducing the disease infection.

Keywords: Chickpea, Salycilic acid, Fungal diseases, *Fusarium roseum*, SAR.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most popular vegetables in many regions of the world [1,2]. Chickpea is valued for its nutritive seed composition which is high in protein content. Increasingly, it is also used as a substitute for animal protein [3].

In Algeria, chickpea is the second food legume after the soybean. A progressive change in its productivity has been happened between 1990-2013, its total production between 1990 and 2013 came 34.980 tons [4]. However because the chickpea crop is highly affected by biotic constraints, a significant instability and decrement in the production of chickpea has appeared in recent years [5, 6].

Fusarium pathogens are among the most aggressive fungus causing wilts and rots on the chickpea. Despite the economic losses they cause, the control of these pathogens remains limited to prophylactic measures; soil disinfection is never complete because firstly of the difficulty of its realization [7] and secondly, the induction of resistant strains.

Salicylic acid (SA) application is known as a plant hormone that has the role of signal in responses of defense, whose the acquired systemic resistance [8]. SA participates in the regulation of the physiological process and the activation of the plant responses toward various stresses (fungi attacks, wounds, bacteria [9, 10]. SA stimulates the expression of the production genes of the PR proteins [11-13], it also adjusts the cell death combined with the hypersensitive response by activation of the peroxidation of lipids and the generation of free radicals, as well as the activation or the inhibition of the antioxidant enzymes, for several plants [14-18].

The main objective of this work was to suggest the Salicylic acid to control the *Fusarium roseum* and the resistance of chickpea during the biotic stress.

MATERIALS AND METHODS

Biological material

The isolated samples of *Fusarium roseum* used in this study were obtained from chickpea plants that showing the symptoms of wilting collar. It was confirmed as *Fusarium roseum* (INRA, Constantine, Algeria). The isolated fungus was grown and conserved on PDA medium at 25 ° C.

Seeds SA treatments

The variety chickpea seeds used in this study were provided by INRA, Constantine, Algeria. The experimental material comprised two chickpea genotypes; FLIP 90-13C and GHAB5. Seeds were treated for 24 hours with different concentrations of SA (100 mg/l, 150 mg/l, 200 mg/l and 250 mg/l) or with distilled water [19].

Test efficiency of Salicylic acid in the inhibition of the growth of *Fusarium roseum* in PDA medium

Standard solution of Salicylic acid was prepared. Certain quantities of this solution were mixed up in a milieu of PDA according to the used concentrations. Then, the obtained solutions were distributed on Petri dishes with three replicates for each concentration. After the hardening of the milieus, the center of each Petri dish was vaccinated with a disk of 0.5 cm that was taken from the edge of a growing colony of *Fusarium roseum*. All dishes were grown in a temperature of 25 ° C. When the growth of pathogens attained the edges of dishes in the control treatment, the rate of diameter growth of the fungus pathogen was calculated according to the following equation:

$$\% \text{ of inhibition} = \frac{(\text{Growth average of control} - \text{Growth average of treatment})}{\text{Growth average of control}} \times 100$$

Experiments of pots

Two genotypes of chickpea (ILC 3279 and FLIP 8555) were used in this experiment. Seeds were sterilized with Sodium Hypochlorite (0.5 %) and then were washed with distilled water. These seeds were immersed for 24 hours in different concentrations of Salicylic acid (100, 150, 200, 250 mg/l). The control seeds were immersed in distilled water. After that, all seeds were put in Petri dishes in a distilled milieu of growth for a period of three or four days [7]). After the growth, seeds were transformed to pots filled with distilled soils which were previously vaccinated with *Fusarium roseum*. Treatments were prepared as the following, with three replicates for each treatment:

Treated seeds with 100 mg/l of SA planted in soil contaminated with fungi.

Treated seeds with 150 mg/l of SA planted in soil contaminated with fungi.

Treated seeds with 200 mg/l of SA planted in soil contaminated with fungi.

Treated seeds with 250 mg/l of SA planted in soil contaminated with fungi.

When the symptoms of *Fusarium roseum* appeared on the plants such as the yellowing of leaves and stems, wilting of plants, and coloration of roots with black, the percentage of infection was measured for each genotype compared with the healthy plants. The following equation was used:

$$\% \text{ of infection} = \frac{\text{Degree of infection of control plants} - \text{Degree of infection of treated plants}}{\text{Degree of infection of control plants}} \times 100$$

All data were obtained in triplicate and results were presented as means of three values.

RESULTS AND DISCUSSION

Effect of SA on the percentage of inhibition of *Fusarium roseum* in the milieu of PDA

Results of the figures 1 and 2 showed that the high concentration of SA (200 and 250 mg/l) affected considerably in decreasing the percentage of the growth of *Fusarium roseum*. The percentage of inhibition was very high at these concentrations and attained 50% 40% respectively. The percentage of inhibition at the concentration 150 mg/l was 27% which differed from the control and from 100 mg/l (34%). The cause of this inhibition could be attributed to the high concentration of SA in the nutritive milieu (PDA). The increase of SA and its accumulation in the body of living things might intoxicate them as the SA is transformed to a toxic composition (Glucoside SAG) which could not be excluded by living organisms [20]. Our results are consistent with those of Kataria *et al* [21] which used the chemical solutions stimulating the resistance in plants such as Salicylic Acid 5- nitro and O-Acetyl Salicylic Acid. Kataria *et al* [21] demonstrated that *Fusarium roseum* could be inhibited by those solutions at concentration 2.5, 5, and 10 ml. Ozgonen *et al* [22] showed that Salicylic Acid has the ability to inhibit the fungus pathogen *Fusarium oxysporum* at the concentration 0.6 M μ . Also, the work of Naji and Jawadayn [20] indicated that the high concentration of SA may completely inhibit the growth of *Rhizoctonia solani* in PDA.

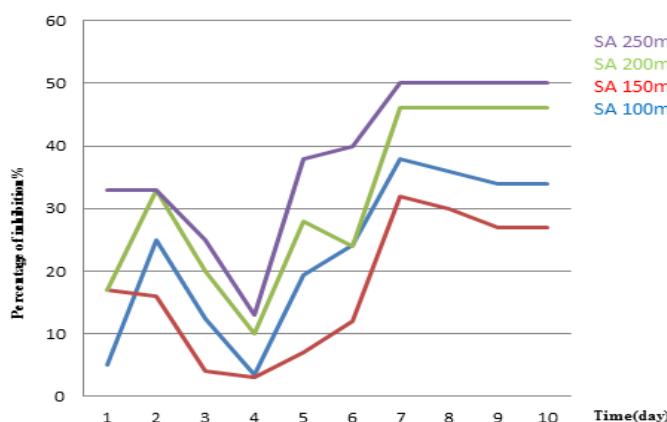


Figure 1: Inhibition percentage of *Fusarium roseum* at different concentrations of Salicylic Acid.

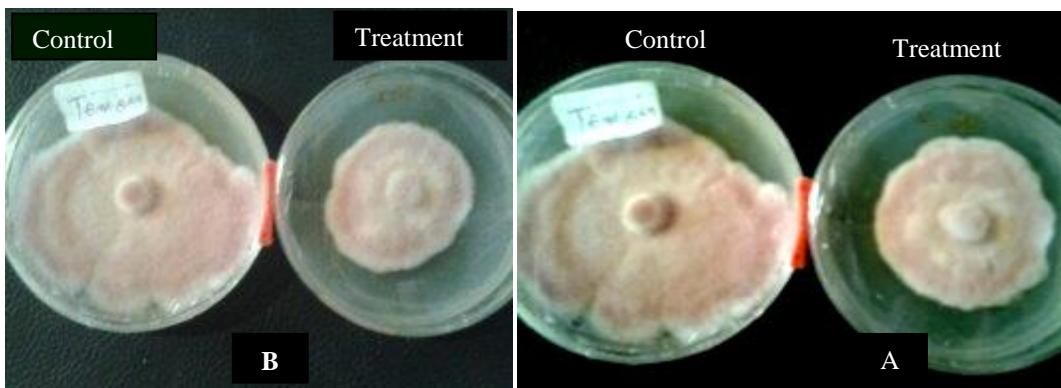


Figure 2: Inhibitory effect of Salicylic Acid of the mycelial growth of *Fusarium roseum*

A- Colony of *Fusarium roseum* at 200 mg/l (SA)
B- Colony of *Fusarium roseum* at 250 mg/l (SA)

Effect of SA treatment on the percentage of infection by *Fusarium roseum* in pots

Data showed in the figures 3 and 4 indicate that all concentration of SA highly decreased the percentage of infection by *Fusarium roseum*. The percentage of infection were high in the concentration 100 and 150 mg/l for the genotype FLIP 90-13C and were 100% and 97.47% respectively. These percentages were somewhat similar to those observed in the control which attained 100%. While in the concentration 200 and 250 mg/l, the percentage of infections were low (61.9%, 38.09% respectively).

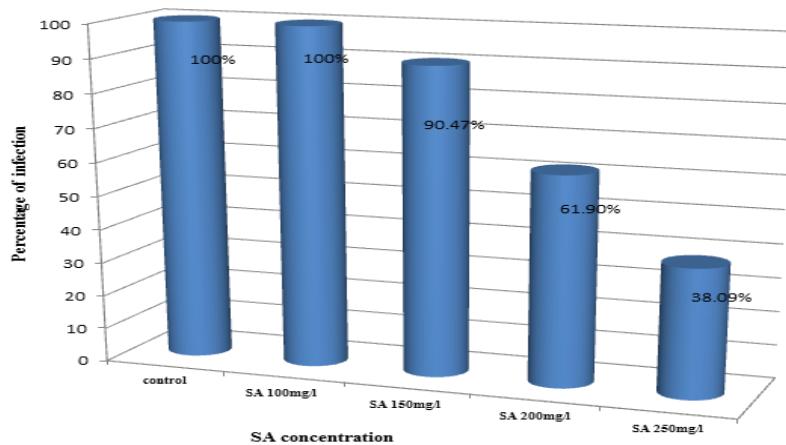


Figure 3: Salicylic acid effect on the percentage inhibition of *Fusarium roseum* in FLIP 90-13C

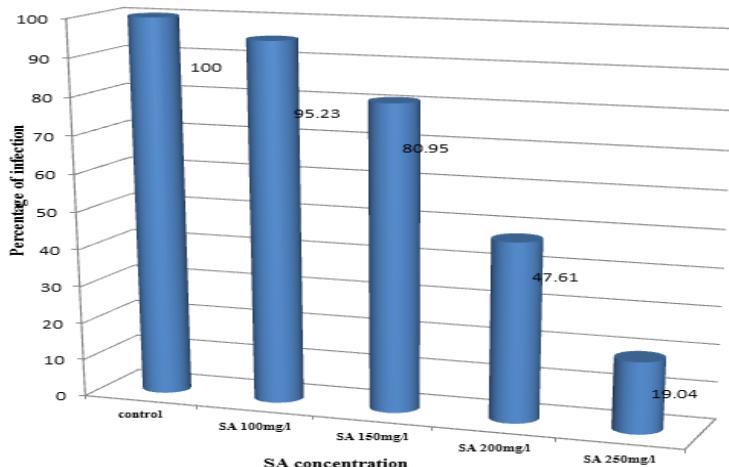


Figure 4: Salicylic acid effect on the percentage inhibition of *Fusarium roseum* in GHAB 5

For the genotype GHAB 5, the lowest values of infection were 47.61% and 19.04% which observed in the concentrations 200 and 250 mg/l respectively, in comparison with the concentration 100 and 150 mg/l in which the percentage of infection were 95.23% and 80.95% respectively. This can be explained by the efficiency of SA in resisting the fungus *Fusarium roseum* by limiting its development as the *Fusarium roseum* accumulate in the xylem vessels and prevent the passage of water to different parts causing the wilt of the plant.

Salicylic Acid is considered as one of the plant hormones [23] which play a substantial role in decreasing the damages results from the pathogens such as the fungus. Additionally, SA is an important factor provoking the Systematic Acquired Resistance (SAR) against the different pathogens [24].

Several studies had demonstrated that SA might stimulate the resistances in plants such the *Arabidopsis* [25] and this because SA might stimulate a group of genes responsible for the mechanisms of resistance in plants [26].

The results of the present study were consistent with those of [20] which they treated the legumes by SA and observed that the resistances against *Rhizoctonia solani* were increased. Also, Juber and Hasson (2006) [27] showed that the percentage of infection by *Rhizoctonia solani* in potato tubers were 100% when treated by the fungi, while this percentages decreased in the treated plants by Salicylic Acid.

CONCLUSION

The present study could be considered the first in Algeria in which the hormone of Salicylic Acid was used to stimulate the Systematic Acquired Resistance (SAR) against the fungus pathogen *Fusarium roseum* in the Chickpea plants. Results of researches on other substances showed the presence of stimulating effects in the Systematic Acquired Resistance in the Chickpea plants which can decrease the intensity of disease by other funguses.

Based on the present results, it could advice to utilize the Salicylic Acid as stimulating agent to decrease the degree of infection by Fusarium diseases by immersing the seeds of Chickpea in the mentioned concentration for 24 hours before the planting. Also, it could sprinkle the plantlets before a sufficient period of the infection by pathogens. This can significantly contribute in limiting the appearance and development of diseases.

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