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Boukraâ.D

Laboratory for Research on Biological Systems and Geomatics, Faculty of Sciences Nature and Life. University of Mascara. P.O. Box 305. Mascara, Algeria.

Belabid.L

Laboratory for Research on Biological Systems and Geomatics, Faculty of Sciences Nature and Life. University of Mascara. P.O. Box 305. Mascara, Algeria.

Benabdelli.K

Laboratory for Research on Biological Systems and Geomatics, Faculty of Sciences Nature and Life. University of Mascara. P.O. Box 305. Mascara, Algeria.

Bennabi.F

Laboratory of Plant Physiology, Department of Biology, Faculty of Sciences, University of Oran, Algeria

The effect of the Salicylic Acid on the variability of phenolic compounds, during the germination and the seedling of chickpea (*Cicer arietinum* L.), after inoculation by mushrooms

Boukraa.D, Belabid.L, Benabdelli.K, Bennabi.F

ABSTRACT

In Algeria, the growing of chickpea is subject to a certain number of mushrooms that limit its production, its development and its expansion.

The present study is carried out in order to show the effect of the salicylic acid on the induction of the resistance with the chickpea (*Cicer arietinum* L.), in phase of germination and during its growth, under biotic stress conditions. In this frame, a test was lead in controlled conditions on a sensitive variety of chickpea (ILC 3279) pretreated in the salicylic acid (T, 0,05mM and 0,5mM), then inoculated by the *Pythium* spp. (factor of melt of seeding) and *Fusarium.oxysporum* f.sp. *ciceris* (factor of vascular wilt of the chickpea).

During this study, were also studied the precocity, the kinetics and the final rate of germination of seeds, the number of the cultivated and necrosis plants, the mobilization of certain reserves and the antioxidant capacity.

Obtained results show that the application of the salicylic acid causes an increase of the germination ability of the chickpea seeds and the plants in growth against a decrease of necrosis plants rates as well as an increase of the rate of chlorophyll, of proteins, of polyphenol is noticed going along with a growth in the antioxidant capacity, according to the studied stage (analyzed seeds and plants).

To conclude, we can suggest that the application of the salicylic acid at (0,05 mM) may urge on the resistance of chickpea, during the biotic stress in the course of the germination and the growth, by increasing the report of some protective biochemical molecules, playing a role in the induction of resistance.

Keywords: Salicylic acid, Chickpea, *Fusarium* spp., *Pythium* spp., germination, polyphenol.

1. Introduction

The eating legumes, from the area they occupy, represent an important place in the agrarian system and the food – economics in many countries all over the world.

Indeed, these legumes have a particular importance for agriculture, for the biological characteristics in the air nitrogen fixation by bacteria and its capacity of adaptation to difficult pedo-climatic conditions as well as their weak food-technical demands. From a nutritional point of view, the legumes, given their wealth in proteins, allow to a certain extent to reduce lacks in animal proteins, in the absence of a food balance of populations who tend to eat cereals ^[1,2].

Among these legumes, the chickpea (*Cicer arietinum* L.) is a plant cultivated in Algeria since Antiquity for its seeds, which are consumed as dried legumes ^[3]. Its farming contributes to the fertility of grounds by nitrogen substances; consequently it is used in many countries in rotation with cereals. Indeed, this capacity of using “free” nitrogen, allows them to reduce the production costs, on one hand, and to limit pollution of ground waters by fertilizer nitrates on the other hand.

The farming of chickpea has known a very important development those recent years.

Unfortunately, badly suitable cultural practices and a neglected sanitary aspect allowed a significant development of the wilt caused by *Fusarium oxysporum* Schelcht. Emend. Snyd. & Hans. f. sp. *ciceri* (Padwick) (FOC) in the country ^[4].

Correspondence:**Boukraâ.D**

Laboratory for Research on Biological Systems and Geomatics, Faculty of Sciences Nature and Life. University of Mascara. P.O. Box 305. Mascara, Algeria.

Due to the extent and the severity of the disease, the chickpea production has significantly fall; pushing farmers into giving up the farming sometimes [4].

In Algeria, the chickpea (*Cicer arietinum* L.) is the second provided eating legume after the broad bean. Because of the great wealth of seeds and leaves in plant proteins, they represent in poor countries an important food source [5, 6]. More than 120,000 tons are imported each year by Algeria [7].

The salicylic acid (SA), very widely spread in plants, is considered as a plant hormone of another phenolic that has the role of a signal in responses of defense, whose the acquired systemic resistance (SAR) [8]. It was found in the leaves and reproductive organs of thirty four types of an agronomical importance [9]. It participates in the regulation of the physiological process and the activation of the plants' response toward various stresses (attacks from mushrooms, bacteria, virus, wounds, UV, ozone...) [10, 11]. The Salicylic Acid stimulates the expression of the production genes of the PR proteins [12, 13], it is a transducer and a messenger. It also adjusts the cell death combined with the hypersensitive response by the activation of the peroxydation of lipids and the generation of free stems, as well as the activation or the inhibition of the antioxidant enzymes, for several plants [14, 15, 16, 17, 18, 19].

The objective of the present work is to suggest the salicylic acid to control two fungal diseases of chickpea, during germination and development, as well as the analysis of certain implied biochemical parameters.

2. Materials and methods

2.1 Seeds SA treatments

Salicylic acid (SA; 2-hydroxybenzoic acid) was initially dissolved in 1 ml ethanol and concentrations of 0,05 and 0,50 mM (pH 6,0-6,5) were made up with distilled water [18].

Seeds were treated for 8 hours with distilled water (control), or with the solutions of 0, 05 and 0, 5 mM SA.

2.2 Seed germination

Control and treated seeds were inoculated with *pythium.sp* and distributed to germinate in Petri dishes on two filter-paper discs; each dish contains 50 seeds and sprayed with 20 ml of distilled water. The dishes are put in an incubator at 25 °C. Germination is spotted by the output of radicle from the integuments as described by [20, 21]. Germinated grains are counted, every 24 hours. The test ends when after two successive counts no germination is registered. After 96 h, germinated grain of each dish is taken for biochemical analyses.

2.3 Seedling growth

The second lot of control and treated seeds was raised in earthen pots. At vegetative state, the seedlings were transplanted after 30 days into clay pots (35 cm diameter) containing sterilized sandy loamy soil (1:2 w/w). All plants were inoculated with the FOC and were irrigated with nutritive solution combined to salicylic acid doses (0- 0, 05 and 0,5mM SA).

Three replicates (5 pots and each pot contained 2 plants) were used for each observation under each treatment. Developed plants were counted, the shoot elongation was measured, the number of leafs and the number of necrosis

plant were counted. Vegetative parts were taken for biochemical analyses.

The *Pythium.sp* and *Fusarium. Oxysporum.sp.* were procured from the Agricultural laboratory of University of Mascara.

3. Biochemical analysis

3.1 Preparation of the methanol extracts

For the extraction, about 5.0 g of fresh plant material were homogenized with 10 ml water/methanol (1:1) under magnetic stirring at 4 °C for 20 min. After centrifugation of the mixture (15 min at 4 °C, 4000 xg), the resulting pellet was extracted twice following the same protocol. The supernatants were collected, pooled and centrifuged. The obtained extract was concentrated by rotary evaporation at 30 °C. [22].

The residue was dissolved in deionized water and used for the following analyses:

Chlorophyll, Total phenol, flavonoids, tannins, antioxidant activity.

- 1- Chlorophyll contents were measured using the method of [23]. Fresh plant material (1g) was roughly homogenized in mortar by keeping the temperature at 2 °C in dark condition and extraction was carried out using 90% acetone, with addition of a pinch of magnesium carbonate, to protect and stabilize the chlorophylls. This extract was centrifuged. The residue was washed thoroughly 2-3 times with 90% acetone, collecting all the washings and the final volume was made to 100 ml with 90% acetone. Absorbance of chlorophyll 'a' and 'b' was recorded using double beam UV-Visible spectrophotometer (Shimadzu, Germany), at 663 and 645 nm using 90 % acetone as blank. Following formulae were used to determine the chlorophylls content.

$$\text{Chlorophyll 'a'} = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Chlorophyll 'b'} = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$\text{Total chlorophyll 'Z'} (a + b) = 8.02 \times A_{663} + 20.20 \times A_{645}$$

- 2- Total phenol content was determined using the method of (24). (50 µL) of methanolic extract were put in test tubes and the volume was made up to 500 µL using distilled water. Then, 250 µL of Folin-Ciocalteu reagent was added into the test tube followed by 1.25 mL of sodium carbonate solution. The tubes were vortexed before incubated in the dark for 40 min. Absorbance was read at 725 nm using spectrophotometer (Shimadzu). Total phenolic content of plants was expressed as mg gallic acid equivalents (GAE) g_{1FW} through the calibration curve with gallic acid.
- 3- Total flavonoid contents were measured according to a colorimetric assay [22]. At 250 µl methanolic extract was added to 10 ml volumetric flask containing 1ml of didistillate waters, 75 µl of NaNO₂ (5%) was added to the flask. After 5 min, 75 µl of AlCl₃ (10%) was added. At 6 min, 500 µl of NaOH (1N) was added to the mixture. Immediately, the solution was diluted by adding 2.5 ml ddH₂O and mixed thoroughly. Absorbance of the mixture, was determined at 510 nm

versus the prepared blank. Total flavonoid contents were expressed as mg catechin equivalents (CE)/ g dry weight (dw).

- 4- Tannins content: Using the method of [25], for determination of condensed tannin. 1 ml of methanolic extract was added to 2 ml of vanillin solution (1%) in H₂SO₄ (70%). The mixture was placed in bain -marie for 15 mn at 20 °C, in dark. Absorbance of the mixture was determined at 500 nm with spectrophotomètre.

Condensed tannin contents were expressed as mg cyanidines equivalents (CE)/ g fresh weight (dw).

- 5- Antioxidant activity was measured by DPPH determination [22]. To 2, 9 ml de DPPH à 0,004% (P/V) in methanol- H₂O (8: 2), added to 100 µl of the plant methanolic extract was added. The mixture was shaken and allowed to stand at 20 °C in dark for 30 minutes. After the decrease in absorbance, the resulting solution was monitored at 517 nm. The DPPH radical scavenging activity of phenolic compounds was expressed as mg /100 g of dry matter and as mg /100 ml of VCEAC in 30 minutes. The control solution was consisted by 100 µl of methanol and 2.90 ml of DPPH solution. The radical solution was prepared daily. The percentage inhibition of the DPPH radical (IP50) by the samples was calculated using the formula: $IP50 = [(A_c - A_x)/A_c] \times 100\%$

- 6- Soluble protein content was determined by the method of Bradford (1976) [26].

4. Statistical analysis

Results are presented as mean ± standard Error; statistical analyses of experimental result were subjected to analysis of variance with student test. Significant difference was statistically considered at the level of $P < 0.05$.

5. Results and discussion

5.1 Effect of the Salicylic Acid (SA) on the germination

According to the pictures 01 and 02, seeds of chickpea were germinated since 24 h, either for the control seeds or the seeds treated with the salicylic acid. Except for the presence of 1 mM of the SA, no germination was noticed.

But the presence of the *Pythium* spp., has a depressive effect on the kinetics and the germination rate of the control seeds, during the 72 h, respectively of (21, 6% to 23, 5%). On the other hand, the seeds pre-treated with the two concentrations of the SA have reached a very high germination rate, during the 72 h, respectively of (30.9%, 39%, 55% until 57.7%) with 0.05 mM AS and (27.2%, 37%, 40% and 57,7). But this rate has fallen in 96 h by the root of the control seeds at (0%) and 23, 4% with 0.5 mM SA. Unlike the seeds treated with 0,05mM, the germination speed has only slowed down. *The (0,05mM SA) dose had a positively significant effect than (0,5mM SA) (P < 0.05), in the course of the germination.*

5.2 Effect of the SA on the growth (Pic. 01)

The treatment of seeds then the watering of plants with the different doses of the SA, had shown a significant effect on parameters of growth of the chickpea, infected with the

FOC, according to the figure 01. The percentage of the developed plants was estimated in relation to the control ones. The number of developed plants is much higher following the application of the SA doses (0, 05 mM and 0, 5 mM), respectively of (55% - 44 %) in comparison with the control plants that do not exceed (25%). In parallel, we have noticed a very reduced rate of the necrosis plants, with the two doses of the SA (28, 2% - 14, 7%) compared with the witness plants (43, 1%) (Fig. 01).

Also, the measurements made on the strained muscle of the stems and the number of leaves had shown a positive effect of the treatment with the different doses of the SA (26- 24, 2 and 13, 7 -10, 9 cm and), in comparison with the development of the control plants (8, 2 and 20, 7 cm). With the intention of characterizing the effect of the treatments made on the vegetable state of the chickpea seedlings, weightings of the seedlings vegetation of each treatment were carried out at the harvesting (Pic. 01). The maximum weights of the vegetable parts were indicated for the plants watered with the SA, in comparison with the control plants (0,853 g). Nevertheless, variations were noted for the different doses, (1,604 g) with 0,05mM SA, while with 0, 5 mM SA the weights were less (1,032 g) (Fig. 01).

The doses (0,05Mm SA) had a positively significant effect than (0,5mM SA) (P < 0.05), in the course of development.

5.3 Effect of the Salicylic Acid on the biochemical parameters

In order to characterize the effect of the SA on the nutritional quality and identify some interfering molecules in the activation of the resistance in seeds and plants, against the *pythium.sp* and the *FOC*, the following results were observed.

5.4 Photosynthetic Pigments (Chlorophyll a, b and z or total chlorophyll) Contents

Rates of different Photosynthetic Pigments (Chlorophyll z, b, a) are illustrated in the (Pic. 03.). A concentration of chlorophyll was noted in the plants watered with the SA compared with the control ones. But we have noticed that the dose 0, 05 mM SA raise significantly the chlorophyll (z, b, a) respectively (192, 8 - 80, 7- 49, 2 mg) while at the application of 0, 5mM SA, the chlorophyll reach (91, 6 - 59, 8 - 45, 8 mg). On the other hand, for the control plants, this rate is less and does not exceed (83, 1-53, 3-34, 4 mg). Also, **chl b** contents were higher than the **chl a** contents.

5.5 Proteins Contents: The quantitative analysis of the efficiency of the treatments made on the proteins contents were shown in the (Pic. 04). In general, a great accumulation of proteins was noticed in seeds in relation to the plants. Following the application of the SA, these contents have respectively reached in seeds and plants : (6,68 mg - 3,36 mg) with 0,05 mM SA and (6,54m -3,04mg) with 0,5mM SA. While the less noted contents are in the control seeds or plants (4, 2 mg -1, 66 mg).

5.6 Polyphenol content

According to the Pic.02, the phénolique compounds concentrations are significantly high either in the seeds or in the plants no- treated with the SA. The polyphenol rate is much reduced with 0,05mM of SA, it reaches respectively in seeds and plants (1, 78 - 79, 8 mg). While, these rates reach

(1, 7 - 109, 4 mg) with 0, 5mM, in comparison with the control ones (3, 56 - 127, 6 mg) ((Tab 02).

An analysis of some polyphenols was combined with biochemical analysis:

5.7 Flavonoids Rate: A remarkable increase of flavonoids contents was noticed in plants in relation to the seeds of the chickpea, in the presence or in the absence of the SA. On the other hand, we have noted that the higher rate of this compound is in the non-treated seeds (8, 81 µg) in comparison with the seeds treated with the doses 0,05mM and 0,5mM of the SA respectively (4, 6 and 6, 95 µg). Moreover, in the plants, the flavonoids content reaches its maximum with 0,5mM (989, 1 µg) and with 0,05mM (824 µg) in comparison with the control plants (502, 5 µg) (Tab 02).

5.8 Tannins Rate: The condensed Tannins contents are in a remarkable growth in the chickpea plants treated by the doses of the SA respectively of (0, 29 µg), in comparison with the control ones (0, 22 µg). On the other hand, we have noted that the higher rate of this compound is in the non-treated seeds (3, 1 µg) in comparison with the seeds treated with the doses of 0,05mM and 0,5mM of the SA respectively (2, 5 and 2, 4 µg) (Tab 02).

5.9 The Antioxidant Activity

With the intention of characterizing the antioxidant activity, the calculated values for the treated samples as well as the control ones are shown in the picture 05. The application of 0,5mM of the SA causes a raise in the antioxidant ability, in the seeds and the plants ; (1,57-17,4) in the seeds than 0,05 mM which cause a deficit (4,7-21,7), in comparison with the control seeds and plants (3,7 et 19,14).

6. Discussion:

The role of the SA in the induction of the systemic resistance in plants, following the attack by the phytopathogenic agents, was already proved [27, 28, 31]. We noted following the obtained results, that the Salicylic Acid can contribute towards the protection of the chickpea against certain fungal diseases.

The study of the precocity and of the kinetic of the germination was remarkably checked by the SA, as well as the strained muscle of the stem, in relation with the control seeds. These results tally the results of [29]. The protective power of this molecule is well noted in the treated seeds, with the different concentrations and subject to the *Pythium.sp.* infection. We can also note that the weaker dose (0,05mM) was remarkably the most reliable, either on the precocity to the germination, or on the speed, the percentage and the protection of the germinated seeds. On the contrary, the dose of 0,5mM was less effective on the precocity to the germination and the protection of the germinated seeds, that presents the rate of the germinated and infected seeds the most raised. Except from the higher dose of (1mM), we note a complete inhibition of the germination.

Moreover, we can observe the of the impact of the SA on the yield of the treated plants in relation with the non treated plants. Indeed, the infection of the plants with the *FOC* could create losses on the number of non-treated plants, leaves had developed chlorosis and at the end of the experiment, plants were died in big majority.

By infecting plants by the *FOC*, this causes lacks at rising, impair of the growth, necrosis of plants or a bad quality of seeds [30, 31, 32]. The vascular wilt caused by the *FOC*, is characterized by a massive overgrowth of the conductor vessels in which the mushroom sporulates. The microconidies are passively transported by the flow of the xylem and thus can infect the air parts. The presence of mycelium and its conidiums as well as the local responses of the plant (creation of tyloses, resins) will cause the blocking of the water transport in vessels and consequently signs of wilt that is the result of a severe hydric stress [33]. [12, 13] had shown the power of the SA on the induction of the systemic acquired resistance (SAR), in plants of different pathogenic. On the contrary, a less harmful effect was noticed in the plants treated with the SA, either on the yield or on the growth parameters (lengths of stems, number of leaves and the weight of the fresh and the dry matter). [34, 35] had shown that the SA stimulates the cell division of roots, as well as the increase of the foliar mass and the weight of plants, by stimulating the mitotic system of the apical meristems. Similar results were proved in ric [36, 3, 38]e.

The pathogenic factors reduce generally the synthesis of the chlorophyll or accelerate its degradation, by causing the degeneration of chloroplasts [39, 40]. Measurements of the chlorophyll rates show an increase with the weak dose 0,05mM of the SA against a decrease with the dose 0, 5 mM. These results confirm the results of [39, 41, 42], who had informed that the application of strong doses of the SA contributes to the decrease of the photosynthesis and of the chlorophyll rate. We can note that the effect of the SA on the chlorophyll in the chickpea shall depend on the applied doses.

Results illustrated in the figure (3) and the table (1) show a certain proportionality, but opposite, between the chlorophyll contents and the developed plants percentage for each used dose. The dose of 0,05mM SA indicates a strong accumulation of the chlorophyll that had given the biggest percentage in developed plants and in parallel a less high necrosis rate. Unlike, the accumulation of the chlorophyll was weaker, the number of developed plants was less and the rate of necrosis was higher, with (0,5mM SA). These results suggest that there is a close links with the chlorophyll contents and so the photosynthesis. Moreover, [43] has noted that the roots were very sensitive to the SA, since observations under a microscope had shown that the bristles absorbing roots were deformed as the SA concentration increases. This same author indicates that the SA with weak doses plays a role in the defense against root pathogenic factors in *M. truncatula*, concerning either bacteriums or mushrooms.

A massive production of proteins is remarkably noticed in seeds. But these contents are higher in samples treated and infected in comparison with witnesses. Proteins are involved in the response towards stress, in defense reactions of the plant or in changes of the cell lining. Many authors had informed that the SA plays the role of the signal molecule in the induction of defense proteins (Pr) in contact with parasites [12, 44, 45]. [46] Had noted an accumulation of the PR proteins following the application of the SA in plants. [47] Had shown the influence of the SA on the accumulation of RNA, they also show a grid of regulation of the genes expression and the participation of these extra cell functions in the resistance expression.

The accumulation of the phenolic compounds, in particular flavonoids, was proved in many species and in different situations of biotic stress [48, 49, 50] especially in infected sites and playing a toxic role for pathogenic factors [51, 52]. Their role in the stimulation of the resistance for legumes was proved [53].

The analysis of polyphenols and at the same time of the flavonoids and tannins has shown the accumulation of flavonoids in the plant compared to seeds. This is in connection with the rate of the air biomass very high in the treated plants (Tab 01).

According to (54), in the presence of the salicylic acid, the PAL and TAL activities were respectively utmost at 24 and 72 hours after inoculation. The PAL and TAL activities were more maximized respectively by 75 et 100 μM of the salicylic acid and the synthesis of the phenolic compounds was simultaneous with the enzymatic stimulation.

The phenolic compounds, especially the tannins, can inhibit the infection of the pathogenic factors by increasing the defense mechanisms of the cell linings, by their lignin process [51]. The tannins are largely distributed in leaves, vascular tissues, teguments of seeds (pro anthocyanidins), as well as of flavonoids present in all the compartments of the seed [55, 56, 57].

[58, 59] had indicated that some flavonoids play the role of phytoalexin, which are very much synthesized by the leaves of the plants further to an infection by pathogens or mushrooms. Under the effect of the SA, an increase was noticed during the two stages (Tab 02). [60] had mentioned the significant effect of the isoflavonoids in the decrease of the growth of the pathogens that cause changes in myceliums and disorganization of cells, (5) proposes that the ability of a plant to resist depends on the rate of the polyphenol. [52] had mentioned that the activity of the oxidase polyphenol reaches its maximum further to a treatment of a plant by the *F. oxysporum* and the SA. On the other hand, for the seeds pre-treated by the SA, this one has reduced contents of this compound compared with the control ones (Tab 02).

[61] had reported that the oxidization of the biochemical compounds and their derivatives are involved in the weakening of the resistance of plants towards the pathogenesis.

The study of the antioxidant activity shows a decrease in control seeds and plants, whereas a development was observed in samples treated with different doses of the SA (fig. 05). [62] had noted that the SA plays an essential role in the decrease of the oxidative effects in seeds on germination. Indeed, the polyphenols contribute to the defense against the species reagent to oxygen (ROS) generated under stress conditions. [63] had found that the tolerant genotypes of the chickpea had revealed high contents of polyphenols.

The rise of the antioxidant ability seems have an important link with polyphenols, the growth stage of chickpea as well as the applied doses of the SA. [64] had reported that the antioxidant ability depends on the specie and the stage of the development of the plant. Wile, [65, 66], had mentioned that the SA degrades the antioxidant ability in the plants.

The antioxidant activity can reduce the effect of some free roots (ROS) that can be toxic in significant quantity, by destroying the cell metabolism by the oxidization of lipids, of proteins and nucleotids [67, 68, 69, 64]. Many studies suggest the predominant role of the salicylic acid in the modulation of the response of plants towards abiotic and biotic stresses by induction of the antioxidant ability, which suggests a role of this isoform in the rise of H_2O_2 levels in the chloroplast [70].

The ability of the SA of inhibition of the activity of the Catalase (enzyme which detoxifies the peroxide of the hydrogen), leads to a rise of the rate of the hydrogen peroxide in vivo and result in an oxidative choc towards sites of the attack by the pathogenic or of the treatment with an elicitor [71, 72, 73]. According to [16], the hydrogen peroxide could stimulate the defense genes.

We can notice that the application of the SA by strong doses can decrease on inhibit the germination of the chickpea seeds, 0, 05 mM is the most reliable to be used in order to treat seeds, during the germination and the growth.

We can suggest that the use of the SA can have a protective role against some plant pathogenic mushrooms, during the germination by increasing the percentage of the germinated seeds and protecting the seeds or during the growth by raising the resistance of the developed plants, with preserving some biochemical molecules, indispensable for the plants resistance and growth.

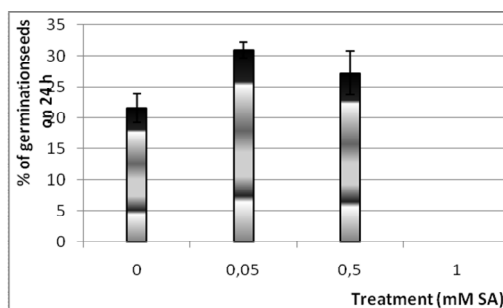


Fig 1: Effect of salicylic acid on seed germination (%) of chickpea (ILC 3279), inoculated with *Pythium.sp* (1, 6.10⁴ conidies/ml), on 24 hour.

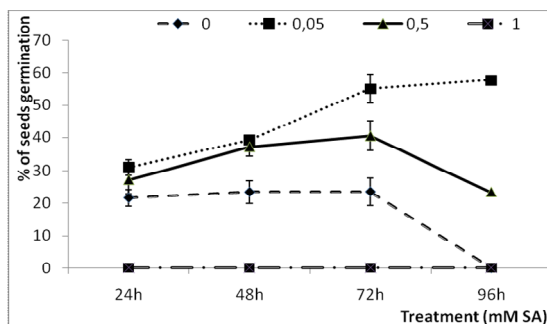


Fig 2: Effect of salicylic acid on kinetic seed germination (%) of chickpea (ILC 3279), inoculated with *Pythium.sp* ($1, 6.10^4$ conidies/ml)

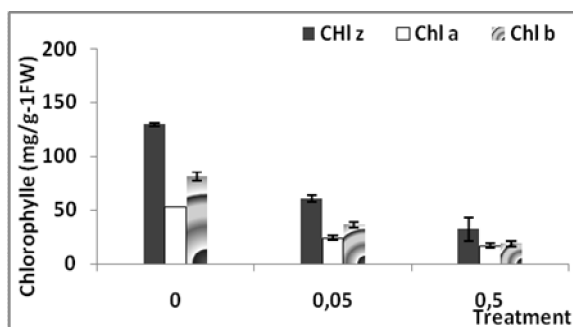


Fig 3: Effect of salicylic acid on chlorophyll content of Chickpea (ILC 3279) inoculated with *Fusarium oxysporium.sp* ($1, 6.10^4$ conidies/ml), during seedling.

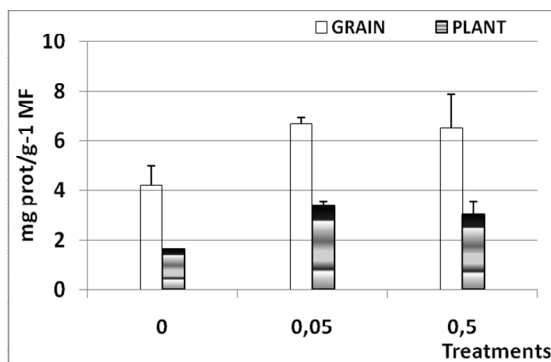


Fig 04: Effect of salicylic acid on protein ($mg/g^{-1}PF$) content of Chickpea (ILC 3279) inoculated with *Fusarium oxysporium.sp* ($1, 6.10^4$ conidies/ml), during seedling

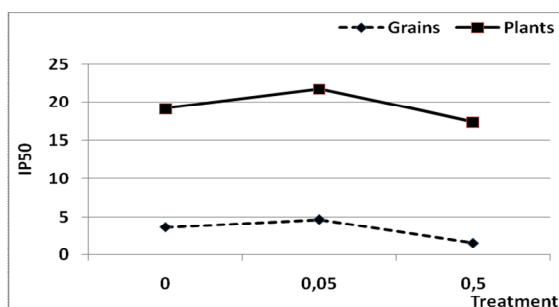


Fig 05: Effect of salicylic acid on antioxidant capacity of Chickpea (ILC 3279) inoculated with Fungi. (The value of IC_{50} reduced represents the higher antioxidant activity).

Table 1: Effect of salicylic acid on growth parameters of chickpea (ILC 3279), inoculated with *Fusarium oxysporium.sp* (1, 6.10⁴ conidies/ml), during seedling.

	T	0,05 mM SA	0,5 mM SA
Developped plants (%)	25	55	44
Necrosis plants (%)	43,1	28,2	14,7
Leaves number	8,2± 2,6	13,7±3,6	10,9±3,9
Shoots length (cm)	20,7±5,3	26±5,7	24,2±5,4
Fresh Weight (g)	1,053±0,1	1,604±0,2	1, 032±0,11
Dry Weight (g)	0,28±0,06	0,61±0,046	0,208±0,06

Table 02: Effect of salicylic acid on Polyphenol content of Chickpea (ILC 3279) inoculated with Fungi (*Fusarium oxysporium.sp* (1, 6.10⁴ conidies/ml)], during germination and seedling.

	Treatments	Polyphenol	Flavonoid (µg/ 100-1g FW)	TC (µg/ 100 ¹ g FW)
	SA (mM)	(mg/100 ¹ g FW)		
Grains	0	3,56 ± 0 ,45	8,81 ± 1,4	3 ,1 ± 0,15
	0,05	1,78 ± 0,23	4,6 ± 0,4	2,5 ±0,5
	0,5	1,7 ± 0,4	6,95 ± 1,5	2,4 ±0,14
Plants	0	127,6 ± 8,8	502,5 ± 93	0,22 ±0,03
	0,05	79,8 ± 18,1	824 ± 123	0,29 ±0,026
	0,5	109,4 ± 6,63	989,1± 39,2	0,29 ±0,031

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