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# Influence of Humic Substances on Cucumber Seeds Storability and Root Rot Diseases Incidence under Salinity Conditions

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

Seed germination is a complex process, which is regulated by many factors including storage. The present study aims at assessing the validity of stored cucumber seeds under stressed-soil. *In-vitro* experiment was conducted to investigate the influence of soaking of stored cucumber (*Cucumis sativus* L.) seeds produced during three consequent years (2015, 2016 & 2017) in five concentrations of humic substances (HS'c) solution (0.3, 0.6, 0.9, 1.2 & 1.5%) for five different intervals (30, 90, 150, 210 & 270 min), on germination percentage (G%), germination velocity (GV) and vigor index (VI). Another In vitro experiment was conducted to assess the direct effect of HS'c on two nutritional media for *Rhizoctonia solani* and *Fusarium solani* mycelial growth, sclerotial productivity & viability, conidia viability. Greenhouse experiment was conducted to assess the effect of soaking cucumber seeds in HS'c and spraying with salicylic acid (SA) (100 and 200mg $L^{-1}$) twice on growth parameters of cucumber seedlings, and controlling the root rot disease caused by *R. solani* and *F. solani* under saline conditions (2.36, 4, 5 & 6 dS $m^{-1}$). The results indicate that

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$T_5$ (0.3% for 210 min.) for 2015 and 2017 and $T_4$ (0.3% for 150 min.) recorded the highest values for all studied parameters. No morphological changes were observed for the highest pathogenic two fungal isolate's mycelial growth on both fungal strains. *R. solani* was affected less than *F. solani* for the presence of different HS'c into nutrient media. 1.5% HS concentration had a reduction effect on the radical growth of fungal isolates, *R. solani* sclerotial production (30%) and viability (53%) and *F. solani* conidial viability (58%). Root rot disease was affected differently by seedling treatments of HS'c and/or SA under the four salinity conditions. The combined treatment; soaking seeds in 0.3% HS (for different period/year) and spraying seedlings with 200 SA had significantly reduced the disease incidence (DI) and disease index of both *R. solani* and *F. solani* specially in the lowest and highest salinity conditions.

*Keywords: Stored cucumber seeds; germination%; Germination velocity; vigor index; humic substances; salicylic acid; saline soil, Rhizoctonia, Fusarium, solani; sclerotia; conidia; root rot disease.*

## 1. INTRODUCTION

Storage is considering the most important factor affecting seed longevity. Many investigators reported that the speed of decline in seed quality is largely dependent on storage, length of storage, type of seeds and seed quality [1]. Cucumber (*Cucumis sativus* L.) seed generally has poor longevity compared to other seeds species with an average P50 of 4.9 years when stored in unregulated conditions in temperature [2]. Cucumber (*Cucumis sativus* L.) is one of the most important and popular vegetable crops belonging to the family *Cucurbitaceae*. Cucumber is a primary source of vitamins and minerals for human body but its caloric and nutritional value is very low [3]. Cucumber plants are considered moderately sensitive to salt stress, since it can tolerate an ECe of about 2.5 $dSm^{-1}$ where yield decreased by 13% with each unit of ECe increase above the threshold value [4].

Soil salinity is a global problem, especially in Egypt's Nile Delta. There is a need to create water supplies as re-using of irrigation drainage waters and at the same time by improving the agricultural productivity of the Nile Delta through subsurface drainage in water-logged lands [5]. Using organic amendments is one of the most important agricultural practices enhancing the plant defense reaction towards biotic and abiotic stress [6].

Cucumber damping off and root rot disease is mainly caused by various pathogens; *Rhizoctonia solani* [7], *Fusarium solani* [8] *F. oxysporum* [9], a number of *Pythium* spp., including *P. aphanidermatum* (Edson) Fitzp., *P. ultimum* Trow, and *P. irregular* Buisman [10]. The excessive use of chemical fungicides controlling root rot disease have hazardous side effects on living organisms, the environment and human health, and overuse could lead to the development of resistance in fungal species [6]. Recently, resistant cultivars, avoidance of primary inoculum development [11] and use of organic amendments used as crop protection strategy [12,6].

Humic acid (HA) is a principle component of HS, which are the major organic constituents of soil (Humus), peat and coal. It is produced by biodegradation of dead organic matter. It is not a single acid; rather, it is a complex mixture of many different acids containing carboxyl and phenol groups so that the mixture is haves functionally as a dibasic acid or, occasionally as a tribasic acid [13]. Both groups of complex organic acids, humic acid (HA) and fulvic acids (FA) have been proven to be involved in three specific chemical reactions; (1) electrostatic attraction (2) complex formation or chelation, and (3) water bridging. HAs and FAs and other humates supplemented into soil by organic amendment can influence, either directly or indirectly, a number of physiological and biochemical processes occurring in plants and soil-borne organisms, especially in the rhizosphere [14,6]. The beneficial effect of HAs and FAs as alternatives to synthesized products in controlling plant diseases, especially Fusarium wilt, is well documented [15,6].

Thus, the objectives of this study are to; *i)* Evaluate the *in-vitro* direct effect of humic substances (HS) assessing HS on:*(1) the mycelial growth under normal ((PDA) medium) and deficient (water–agar medium) nutritional conditions and (2) the sclerotial and conidial germination; ii)* Evaluate the effect of soaking in HS solution on cucumber seeds storability which

alleviate abiotic stress on cucumber plants grown in stressed soils; and *iii)* Assess the effect of HS solutions on root rot disease under saline greenhouse condition.

## 2. MATERIALS AND METHODS

This research work is divided into two experiments to investigate the influence of HS solution on storability of cucumber (*Cucumis sativus* L. cv. Bahi) seeds produced in 2015; 2016 and 2017 by SIEMENS Company, and the response of the soaked cucumber seeds to root rot disease infection under saline conditions (Fig. 1).

### 2.1 Pathogen Isolation and Purification

Pathogen was isolated from cucumber plants showed root rot disease symptoms collected at four locations around Fayoum governorate, Egypt (two fields each) (Zawiet El-Karadsa, Aboxa, El-Menshya and Demo). Pathogens isolation and purification were performed according to Hassan et al. [16]. The isolates were identified based on cultural and morphological characteristics as per Sneh and Auster [17].

### 2.2 Pathogenicity Test of Isolated Fungi

The pathogenicity of twelve strains belonging to the four isolated fungi was assessed using sterilized soil (2 kg/30 $cm^2$ pot). Pots' soil was inoculated with strains grown separately on sand-grounded barley grain culture media at 1% W: W [18]. Pots filled with mixture of sterilized soil and un-inoculated sand-barley medium were used as control. Inoculated pots were kept for 1 week before sowing cucumber seeds (10 seeds / pot). Five replicates for each fungal isolate and control were distributed using complete random design in the experimental greenhouse at Demo, Faculty of Agriculture, Fayoum University. The pre and post emerging damping off percentage were estimated after four and thirty days after sowing.

The highest two pathogenic isolates belonging to two relevant species were used in the next experiment.

### 2.3 Effect of Humic Substances on the Highest Two Pathogenic Fungi Growth

*In vitro* growth inhibition of *Rhizoctonia solani* and *Fusarium solani* was estimated using the poisoned media technique [6] on potato dextrose agar (PDA) and water agar medium (WA). The HS solutions were prepared as described by Afifi et al. [6]. Different HS volumes were added to (45 °C) prepared culture media with final HS'c as follows; 0.3; 0.6; 0.9; 1.2 and 1.5 %. Five replicates / HS'c were inoculated by 5 mm of 7 days old *R. solani* and *F. solani* in the middle of 9 cm petri plates. Inoculated, HS-free plates were

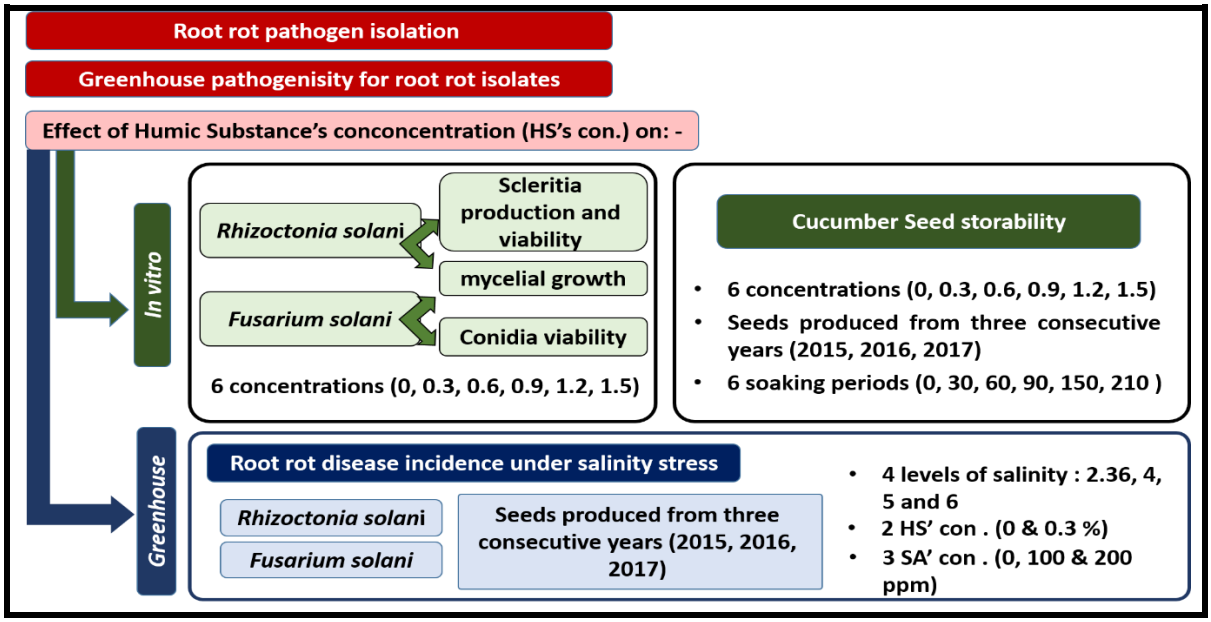



**Fig. 1. Outline of research study**

included as controls. All plates were incubated at 25°C for 10 days. The radial growth of the pathogen was measured until the fungus covered the control plates completely. Inhibition of the pathogen compared to the control was calculated as follows;

$$\text{Reduction of growth} = \frac{\text{growth in control} - \text{growth in treatment}}{\text{growth in control}} \times 100$$ [18]

## 2.4 Effect of Humic Solutions on Sclerotia Production and Viability of *Rhizoctonia solani*

The method described by Harikrishnan and Yang [19] was used. Plates of PDA were amended with the six HS'c (0, 0.3, 0.6, 0.9, 1.2 and 1.5%) as described above. Plates were then inoculated with a 5-mm agar plug from 7days old cultures of *R. solani* isolate in PDA. Inoculated plates were incubated for 4 weeks at 25 ± 2 °C with a 12 h light/dark cycle. Sclerotia from each plate were harvested by sieving (250 µm) under running tap water and then air dried overnight at room temperature. Data on sclerotia weight, number of sclerotia $mg^{-1}$, and viability percentage were calculated.

To calculate sclerotia validity percentage, randomly selected 25 sclerotia / treatment were surface sterilized in 0.05 % sodium hypochlorite solution followed by two washes in sterile distilled water. The sclerotia were then blot dried and plated on PDA. Plates were incubated for two days at 25°C with a 12 h light/dark cycle; germinated sclerotia were counted [19]. A sclerotium was counted viable if it produced visible mycelium. Each treatment was replicated five times. The experiment was repeated once.

## 2.5 Effect of HS Solutions on Conidia Viability of *Fusarium solani*

Suspensions of $5\times10^5$ spores $ml^{-1}$ were prepared from 10-days old *F. solani* culture on PDA medium and mixed with appropriate aliquots of stock aqueous suspensions/solutions of each HS'c to obtain a density and an HS'c of 0 (control), 0.3, 0.6, 0.9, 1.2 and 1.5%. About 50 µl of each mixture were then placed on a microscopic slide that was kept at 20°C in moist micro-chambers consisting of Petri dishes lined with moist filter paper. After 24 hr, the germination percentages of 40 conidia/treatment were measured using light microscope. The conidium was considered germinated when the germ-tube length was at least equal to the conidial diameter. The experiments were replicated twice [12].

## 2.6 Effect of Soaking Cucumber Seeds in Humic Substances for Different Periods on

*In-vitro* experiment was conducted on stored cucumber seeds (which produced 2015; 2016 and 2017 years ago, to investigate the effect of soaking in different HS'c (0.3; 0.6; 0.9; 1.2 and 1.5 %) for five different soaking intervals (30; 90; 150; 210 and 270 min.) as shown in Table 1. on seeds validity. Some germination characters were determined as; germination percent (G%); germination velocity (GV) and vigor index (VI). The stored cucumber seeds were germinated on 10/12/2017; the seeds (10 seeds) were soaked in sterile distilled water and treated with various concentrations of humic acid. All the treated seeds were placed in 10 cm diameter sterile petri dishes containing a thin layer of wet cotton. A 10 ml of each solution was added to each petri dish and transferred to a germinator at 25°C and seeds germinated in distilled water were served as a control. All the germination and early seedling growth parameters were evaluated using the method used by Li [20], with some modifications. Counting germinated seeds started 24 h after sowing every day for 6 days.

A seed was considered to be germinated when plumule and radical emerge from the seeds. Five seedlings from each petri dish randomly selected and radicle and hypocotyl lengths were recorded.

***Germination rate (GR),*** was evaluated as follows;

$$\text{Germination rate (GR)} = \frac{X_n - (X_{n-1})}{Y_n}$$

Where:

$X_n$ is the number of germinated seeds at the $n^{th}$ day,
and $Y_n$ is the number of days from sowing until the $n^{th}$ harvesting time.

$$\text{Germination percent (GP)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$ [21].

Seedlings' *Vigor Index (VI)* was calculated according to formula;

$$\text{Vigor index (VI)} = (\text{mean root length} + \text{means shoot length}) \times (GP)$$ [22]

**Table 1. Humic substance solution concentrations and soaking intervals**

| HS concentration (%) | | Soaking interval (min.) | | | | |
|---|---|---|---|---|---|---|
| | | 30 | 60 | 90 | 150 | 210 |
| Treatments | 0.3 | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
| | 0.6 | $T_6$ | $T_7$ | $T_8$ | $T_9$ | $T_{10}$ |
| | 0.9 | $T_{11}$ | $T_{12}$ | $T_{13}$ | $T_{14}$ | $T_{15}$ |
| | 1.2 | $T_{16}$ | $T_{17}$ | $T_{18}$ | $T_{19}$ | $T_{20}$ |
| | 1.5 | $T_{21}$ | $T_{22}$ | $T_{23}$ | $T_{24}$ | $T_{25}$ |
| | 0 | $T_{26}$ (for 30 min) | | | | |

## 2.7 Influence of Soaking Cucumber Seeds with HS Combined with Soil Salinity, Seedling Spraying by SA on Cucumber Root Rot Disease Incidence

A greenhouse trial was carried out in the Demo experimental greenhouse Fac. Of Agric., Fayoum Univ., located in Fayoum Governorate. The best HS'c efficiency for cucumber germination parameters combined with four salinity levels (2.36, 4, 5 and 6 ds $m^{-1}$) in controlling cucumber root rot disease caused by *Fusarium solani* and *Rhizoctonia solani* and their effect on cucumber growth parameters were evaluated in artificially infested potted soil. Those treatments were sprayed with three levels of salicylic acid (SA) concentrations (0, 100 and 200 mg $L^{-1}$) for three times with two weeks intervals. Cucumber seeds cv. Bahi produced in 2015, 2016, and 2017 were soaked in HS'c at the rate of 0.3 for 150, 30 and 210 min respectively. Five soaked seeds/ four replicates/ treatment were cultivated in four pre-inoculated pots with either *F. solani* or *R. solani* (as described in the pathogenicity test procedure). Four replicates of control treatments / humic substances soaking concentrations mixed with un-fungal-inoculated were used.

Disease incidence was performed form the following formula:

$$\text{Disease Incidence (DI \%)} =$$

$$\text{\% of rotted seeds} + \text{\% of rotted seedlings}$$

Disease severity (DIx) of root rot at the end of the experiment was recorded 45 days after sowing [10], using a rating scale 0-4 as reported by Sallam et al. [23]. Where, 0 = No infection, 1= 1-25% infection, 2 = 26–50% infection, 3 = 51–75% infection, 4= 76-100% infection. The estimation of the disease index percentage was carried out as follows:

$$\text{Disease Index (DIx \%)} = \frac{\sum(n \times 1)+(n \times 2)+\cdots}{t_n} \times 100 \quad [24]$$

Where:

$t_n$: the total number of plants,
n: Number of plants in each group of diseased plants (1, 2, 3 ...).

## 2.8 Statistical Analysis

All experiments were performed twice. Analyses of variance were carried out using the MSTAT-C, 1991 program version 2.10. Fisher LSD test was employed to test for significant differences between treatments at p = 0.05 [25].

## 3. RESULTS AND DISCUSSION

## 3.1 Disease Assessment

Twelve strains belonging to *Rhizoctonia solani*, *Fusarium solani*, *Pythium* spp., and *Macrophomina phaseolina* (4, 4, 2 and 2 respectively) were isolated from infected cucumber plants collected from four different locations at Fayoum Governorate, Egypt (Table 2).

All the fungal isolates had no significant differences in their ability of infecting the cucumber seeds and seedlings. While, they are varied in their disease incidences (Table 3). Where, the $R_1$ and $F_1$ isolates showed the highest significant disease incidence after 4 and 30 days of sowing (Fig. 2). Whereas, the fungal isolate $M_1$ showed the lowest pathogenic for cucumber seeds (Fig. 2-A) and for the total percentage of cucumber rotted seeds and seedlings (Table 3). Other tested isolates had no root rot disease incidence significant difference after 30 days after sowing (Fig. 2-B) (Table 3).

Cucumber root rot disease complex caused by various genera of fungi [26], which mainly caused by *Rhizoctonia solani, Fusarium solani*, *Pythium* spp., and *Macrophomina phaseolina* at Fayoum, Egypt. Where *R. solani* and *F. solani* has the highest significant pathogenic activity to the cucumber seeds and seedlings under greenhouse condition.

**Table 2. Isolated fungi causing cucumber root rot from four different loci at Fayoum governorate, Egypt**

| Location | Isolated strains | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *F. solani* | | *R. solani* | | *M. phaseolina* | | *P. spp* | |
| | + | ++ | + | ++ | + | ++ | + | ++ |
| Zawiet El-Karadsa | 1 | $F_1$ | --- | --- | --- | --- | --- | --- |
| | --- | --- | 1 | $R_1$ | --- | --- | --- | --- |
| Aboxa | 1 | $F_2$ | --- | --- | 1 | $M_1$ | --- | --- |
| | --- | --- | 1 | $R_2$ | --- | --- | --- | --- |
| El-Menshya | 1 | $F_3$ | --- | --- | 1 | $M_2$ | --- | --- |
| | --- | ---- | --- | $R_3$ | --- | --- | 1 | $P_1$ |
| Demo | 1 | $F_4$ | 1 | $R_4$ | --- | --- | --- | --- |
| | --- | --- | --- | --- | --- | --- | 1 | $P_2$ |

[+] *Number of isolated strains;* [++] *Code of isolated strain*

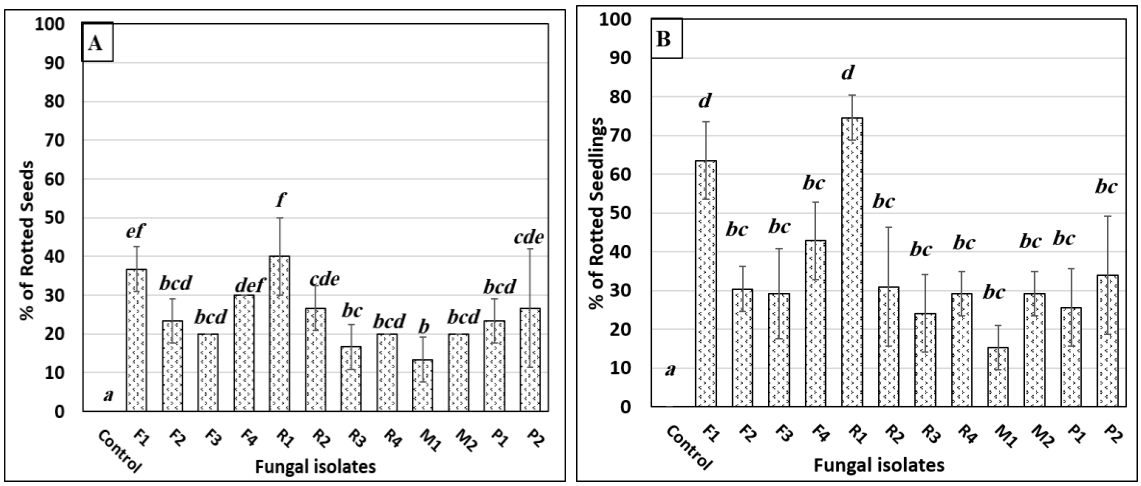



**Fig. 2. Root rot disease incidence in cucumber seeds[*] (A) and seedlings[**] (B) infected by twelve different isolated fungi; F1-F4: *F. solani*, R1-R4: *R. solani*, M1&M2: *M. phaseolina* and P1&P2: *Pythium* spp**

[*]*at 4 days after seed sowing;* [**] *at 30 days after seed sowing; LDS for; pre-emergence seed rot =10.74993, post-emergence seedling root rot=13.70353*

## 3.2 *In-vitro* Fungal Growth Study

No morphological changes are observed for the highest pathogenic two fungal isolate's mycelial growth ($R_1$: *Rhizoctonia solani* and $F_1$: *Fusarium solani*) as a function of different HS'c. Lower HS'c (0.3, 0.6 and 0.9 %) show no inhibitory effect for *R. solani* radical growth comparing with the control one on PDA medium (Fig. 3-A). The inhibitory HS'c effect starts to be observed for the 1.2 and 1.5 concentrations (≈ 7 and 12%, respectively) on PDA medium. However, in the case of WA medium, all of the HS'c have significant radical growth reduction effects on *R. solani* (Fig. 3-A). No significant difference between 0.9 and 1.2% HS'c is observed for *R. solani* mycelial growth on WA medium.

While, *F. solani* radical growth has affected by different concentrations of HS with respect to the control ones (Fig. 3-B) on both cultural media. HS'c of 1.5% has the highest *F. solani* mycelial growth reduction (24.5 and 47 % regarding control treatments, for PDA and WA media respectively), followed by 1.2, 0.9 and 0.6% in a descending reduction order (Fig. 3-B). Whereas, the lowest HS'c (0.3%) has no significant difference for *Fusarium* radical growth reduction on WA medium regarding the control (Fig. 3-B).

Apparently, *R. solani* is less affected compared with *F. solani* for the presence of different HS'c into nutrient media (Fig. 3). Both tested types of cultural media (poor or rich of nutrients) combined with higher concentrations of HS (1.5 and 1.2%) have a reduction effect on the radical

growth of both fungal isolates with respect to control ones and other HS'c. Our findings agree with El-Mohamedy and Ahmed [27], who reported that HS'c has no direct effect on the *F.* solani on PDA. In addition, our research observations are similar to Loffredo et al. [12], using high HS'c in deficient nutritional conditions on the radical growth of *F. oxysporium* f.sp. *melonis*. On the other hand, these findings differ from Abd-El-Kareem [28] ones, which showed that no significant, effect of HS'c on the radical growth of *F. solani* (root infection) and *R. solani* (foliar infection) isolated from bean plants.

### 3.3 *In-vitro R. solani* Sclerotia Production and Viability

The sclerotial production ability of *R. solani* isolate has affected by all HS'c with adverse correlation, where, the higher HS'c shows lower number of produced sclerotia. The 1.5% HS'c reduces the number produced sclerotia of *R. solani* by 30% regarding the control treatment, followed by 1.2 and 0.6% concentrations (≈ 19%). While, the 0.9% HS'c was significantly increased the sclerotia production with reference to 0.6 one (Fig. 4-A).

**Table 3. Cucumber root rot disease incidence (%) (pre- and post-emerging damping off) infected by twelve different isolated fungi; F1-F4: *F. solani*, R1-R4: *R. solani*, M1&M2: *M. phaseolina* and P1&P2: *P.* spp**

| Fungal isolates | Rotted seeds (%) | Rotted seedlings (%) | Total rotted seeds & seedlings (%) |
|---|---|---|---|
| Control | 00.00 $^{f}$ ± 00.00 | 00.00 $^{d}$ ± 00.00 | 00.00 $^{f}$ ± 00.00 |
| F1 | 36.67 $^{ab}$ ± 05.77 | 40.00 $^{a}$ ± 10.00 | 76.67 $^{a}$± 15.77 |
| F2 | 23.33 $^{cde}$ ± 05.77 | 23.33 $^{bc}$ ± 05.77 | 46.66 $^{bc}$ ± 11.54 |
| F3 | 20.00 $^{cde}$ ± 00.00 | 23.33 $^{bc}$ ± 11.55 | 43.33 $^{c}$ ± 11.55 |
| F4 | 30.00 $^{abc}$ ± 00.00 | 30.00 $^{ab}$ ± 10.00 | 60.00 $^{b}$± 10.00 |
| R1 | 40.00 $^{a}$ ± 00.00 | 43.33 $^{a}$ ± 05.77 | 83.33 $^{a}$ ± 05.77 |
| R2 | 26.67 $^{bcd}$ ± 05.77 | 23.33 $^{bc}$ ± 05.77 | 50.00 $^{bc}$ ± 11.54 |
| R3 | 16.67 $^{de}$ ± 05.77 | 20.00 $^{bc}$ ± 00.00 | 36.67 $^{cd}$ ± 05.77 |
| R4 | 20.00 $^{cde}$ ± 00.00 | 23.33 $^{bc}$ ± 05.77 | 43.33 $^{c}$ ± 05.77 |
| M1 | 13.33 $^{e}$ ± 05.77 | 13.33 $^{cd}$ ± 05.77 | 26.66 $^{d}$ ± 11.54 |
| M2 | 20.00 $^{cde}$ ± 00.00 | 23.33 $^{bc}$ ± 05.77 | 43.33 $^{c}$ ± 05.77 |
| P1 | 23.33 $^{cde}$ ± 05.77 | 20.00 $^{bc}$ ± 10.00 | 43.33 $^{c}$ ± 15.77 |
| P2 | 26.67 $^{bcd}$ ± 05.77 | 23.33 $^{bc}$ ± 05.77 | 47.00 $^{bc}$ ± 11.54 |
| LSD | 10.74993 | 13.70353 | 13.96457 |

*Means in columns followed by the same letters are not significantly different according to Fisher LSD test at P = 0.005*

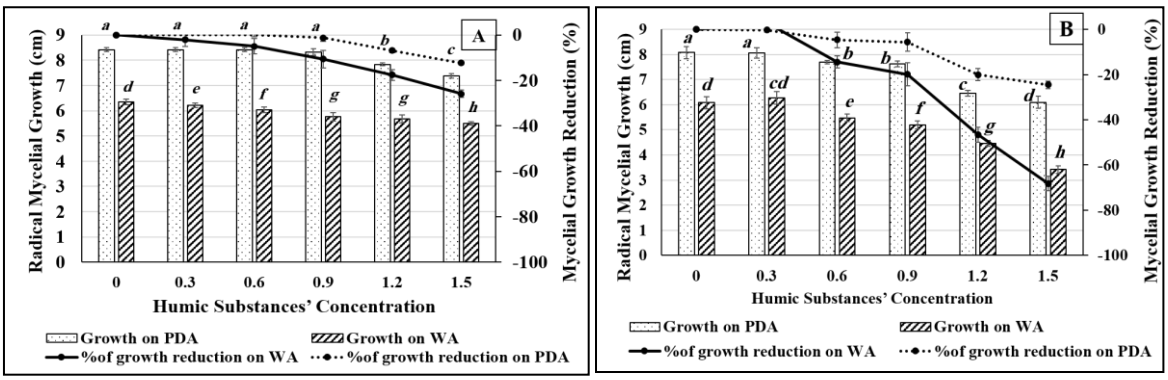



**Fig. 3. Radical mycelial growth and their inhibition of (A) *R. solani* and (B) *F. solani* isolates on PDA and water agar medium amended with humic substances at 0, 0.3, 0.6, 0.9, 1.2 and 1.5% concentration**

*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test (for R. solani: medium x HC= 0.0.13784, medium= 0.05627, HC=0.09747, and for F. solani: medium x HC= 0.22807, medium= 0.09311, HC=0.16127)*

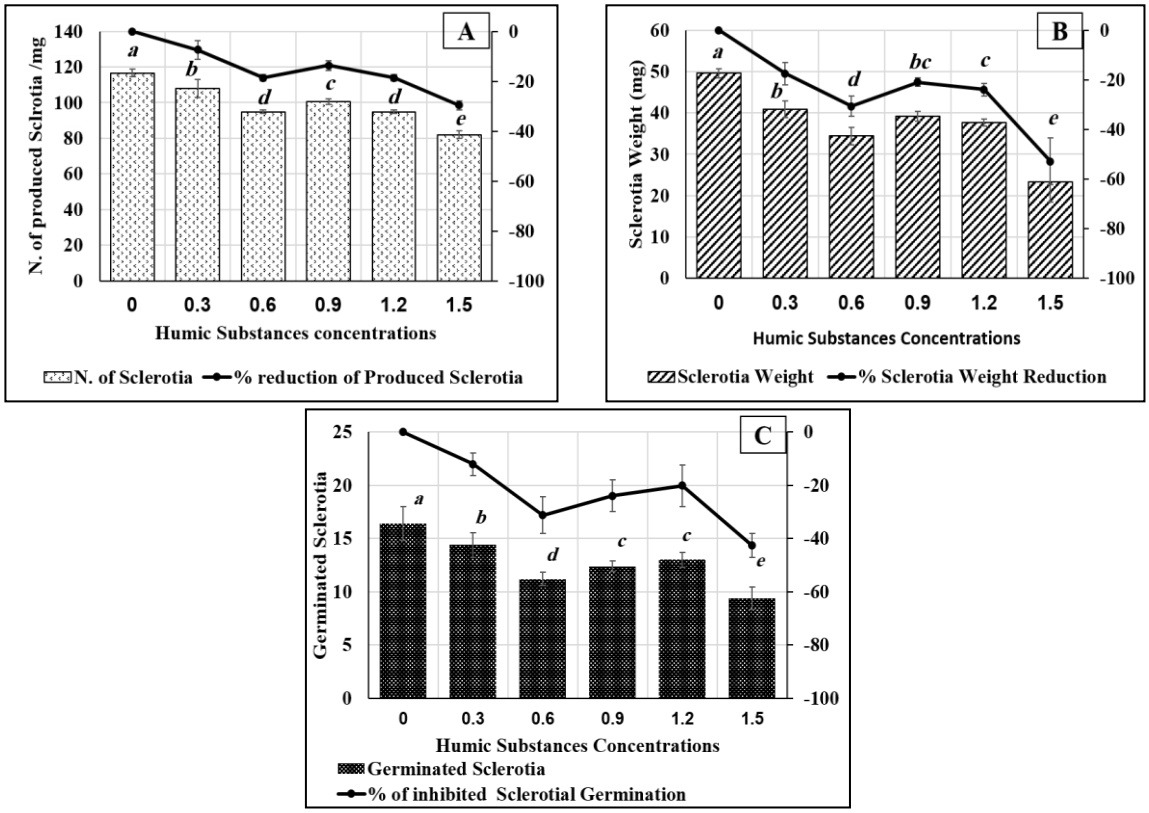



**Fig. 4. Influence of six humic concentration (amended on PDA at 0, 0.3, 0.6, 0.9, 1.2 and 1.5 %) on *R. solani* (A) number (sclerotia mg-1), (B) sclerotia weight (mg) and (C) number of germinated sclerotia**

*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test (sclerotia weight= 2.17512, number of produced sclerotia=1.78196 and number of germinated sclerotia = 0.90652)*

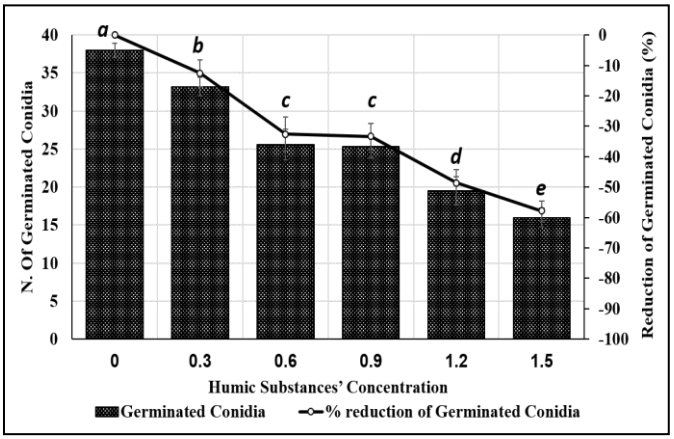



**Fig. 5. Influence of six humic concentration (at 0, 0.3, 0.6, 0.9, 1.2 and 1.5 %) on number of germinated conidia of *F. solani***

*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test (number of germinated sclerotia = 1.35099)*

*R. solani* sclerotial weight is affected by different HS'c but without a constant correlation. Where, the 1.5% HS concentration had the highest significant sclerotial production reduction (≈ 53%) followed by 0.6% one (≈ 31%). There are no significant differences between 0.3, 0.9 and 1.2% concentrations (≈ 18, 21 and 24%) with respect to control one (Fig. 4-B).

The 1.5 and 0.6 HS concentration have the highest significant reduction of germinated sclerotia (≈ 43 and 31% respectively). Where, both 0.9 and 1.2 HS concentrations show no significant differences in between (24 and 20, respectively). Whereas, the 0.3 HS'c has the lowest significant decreasing sclerotial germination effect (12%) with respect to the control treatment (Fig.4-C).

### 3.4 *In-vitro F. solani* Conidial Viability

Data observed at Fig. 5 showed that the increment of HS'c, the decrement germinated conidial number of *F. solani* with reference to control one. Where, 0.3 HS'c showed the lowest decrement percentage of *F. solani* germinated conidia numbers (≈13%), followed by 0.6 and 0.9 HS'c with no significant difference (33% each). Whereas, the 1.5 and 1.2 has the highest reduction of germinated conidial number (≈ 58 and 49 respectively). Obtained results are similar to El-Mohamedy and Ahmed [27], which showed an adverse correlation between the increment of HS concentration and the *F. solani* conidial germination.

Considering *R. solani* one of the important soilborne pathogen depending on sclerotia in its survival in the soil and its saprophytic nature. High levels of humic substances concentrations have a reduction positive effect on mycelia growth and the sclerotia production m number and viability for *R. solani*, that fulfill with Abd-El-Kareem [28] findings; there is an inhibitory effect of humic acid concentrations against linear growth of *R. solani* and*, F. solani*. In addition, this paper indicates the higher HS'c has a direct effect on *F. solani* that reduces its radical mycelial growth and conidial viability. That agrees with El-Mohamedy and Ahmed [27] findings which highlights the effect of HS's on reduction in propagules counts of *Fusarium* spp as well as the incidence and colonization of the pathogen in root of mandarin seedlings. A similar effect had found by Loffredo et al. [12], Afifi et al. [6] the higher HS'c concentration, inhibits highly significantly the radial mycelial growth of *F. oxysporium* f.sp. *melonis* in deficient nutritional conditions and *F. oxysporum* f. sp. *cucumerinum*, causing the cucumber Fusarium wilt disease, respectively.

Despite certain researches, had referred to the absence of direct effect of HS'c on the mycelial growth reduction with *R. solani* ability of degrading HS [29,30]. Filip and Kubát [31] reported that microbial degradation is more HS resistant was associated with increased soil organic matter contents not due to the absence of HS direct effect on microorganisms.

### 3.5 Germination Assay

**Germination percent (G %):** Data in Table 4 shows the influence of stored cucumber soaking (2015; 2016 and 2017) in different humic substances solutions, results show highly significant relationships. However, the mean highest values (4.36; 88.62 and 96.18%) were recorded in 2015; 2016 and 2017, respectively. Among the treatments, the highest values (86.00 followed by 84.67%) were observed with applying $T_4$ (0.3% humic acid for 210 min.) and $T_{19}$ (1.2% of humic acid for 150 min.) and showed a highly significant results over other treatments. This behavior due to the HS plays an important role in seed germination which can be considered as the earlier stimulation induced by the humic molecules according to Eyheraguibel et al. [32], as the HS enter into the seed cells carrying both micronutrients and water, the respiration rate increases and the cell division processes are accelerated improving the growth of the root. Furthermore, the addition of HS on seed treatments improves seed germination and seedling development significantly [33]. However, the excessive concentrations of HS and/or FA can inhibit seed germination at high concentrations and can reduce the growth of young seedlings.

On the other hand, the influence of treatments indicates that the highest values (96.00%) were obtained with using $T_4$ (0.3% humic acid concentration and 210 min.) in 2015; (100%) with applying $T_1$ (0.3% humic acid for 30 min.) in 2016 and (100%) with applying $T_7$ (0.6% humic acid for 90 min.) and $T_{19}$ (1.2% humic acid for 210 min.) in 2017. However, the results obtained were showing also, a highly significant seed germination percent than other treatments. The rates of increasing were 60.00; 4.17 and 4.17% for 2015; 2016 and 2017, respectively as compared with control ($T_0$).

**Germination velocity (GV):** Differences among stored years of cucumber seeds (2015; 2016 and 2017) were statistically a highly significant ($P < 0.001$) in both cases. Also, the averages of germination velocity were (24.00; 85.39 and 108.66) for 2015; 2016 and 2017, respectively. Regarding, the effect of treatments, $T_1$ (0.3 of humic acid for 30 min.) followed by $T_3$ (0.3 of

humic acid for 90 min.) showed the highest value (82.16 and 81.86, respectively) as compared with other treatments and control treatment. These results were attributing to the effect of seeds priming on germination percent indicated that the germination increased in primed seeds due to some metabolism and biochemical changes during priming. For example, in the seeds part of the protein and carbohydrates are broken due to enzyme activity and the hydrolysis reaction. This process resulted in rapid germination and hence seedling emergence can be improved according to Andoh and Kobata [34].

Results regarding the influence of interaction between stored years and treatments, as shown in Table 4, the findings were very similar, however, the highest values 89.35; 106.58 and 117.67) were recorded by $T_4$; $T_1$ and $T_7$ (0.6% of humic acid for 60 min.) for 2015; 2016 and 2017, respectively. The rates of increasing were 206.31; 40.16 and 14.78 for 2015; 2016 and 2017, respectively. Similarly, the results of statistical analysis indicated that there is a highly significant as a result of this interaction. These findings were being explained with increasing concentration of humic acid and soaked interval increased dynamic reserve of seeds. This is indicating better transport of storage materials of seed to vegetative organs.

**Vigor index (VI):** It is clear from the results in Table 4 that the interaction between stored years of cucumber seeds and treatments was highly significant, meaning that the cucumber seeds responded differently at humic acid concentration with soaked interval. This is shown by the significant differences ($p < 0.001$) in vigor index. Different HS'c and intervals had different effects on seedling vigor index, trend of seedling vigor index across different treatments revealed that the greatest seedling vigor index (609.90 and 559.89) in seeds occurred when seeds soaked with concentration 0.6% of humic acid for 150 mins. ($T_5$) and 0.6% of humic acid for 150 min. ($T_8$).

Regardless of treatments, the highest average mean of vigor index (430.86; 393.84 and 97.00) was obtained from stored 2016; 2017 and 2015 cucumber seeds as shown in Table 4. On the other side the influence of treatments showed that the highest values (846.56; 725.86 and 563.04) were obtained with application of $T_3$ (0.3% of humic acid for 90 min.); $T_5$ (0.3% of humic acid for 210 min.) and $T_4$ (0.3 of humic acid for 150 min.) for 2016; 2017 and 2015, respectively**.** HS can be positively effect on the growth of tomato seedlings grown in different environments [35]. In addition, the findings of Asgharipour and Rafiei [36] which indicated that the positive effects of different solution of humic acid on germination and plant growth of seedlings can be due to better water absorption and transport of the stored materials to the roots and shoot growth as well as hormone-like activity of this substance.

The results indicate for occurrence depressing the rates of increasing for vigor index from 2015 to 2017. However, were 203.79; 55.63 and 41.24 for 2015; 2016 and 2017, respectively.

Such positive effects of humic acid on plant growth is a concerned dependence phenomenon and may be due to hormone-like activity of humic acid on cellular respiration, photosynthesis, membrane periment ability of root cells, protein synthesis and various enzymatic reactions [37]. These results are agreed with studies of David et al., 1994), however, the immersion of seeds in a sodium humate solution was reported to increase germination, water absorption, and respiration. Generally, this trail revealed that different concentration levels of humic acid and soaking intervals had a significant effect on seed storage use efficiency.

## 3.6 Effect of HS'c and SA on the Root Rot Disease Incidence

### a) Disease incidence of *R. solani*

Cucumber root rot disease incidence caused by *R. solani* has positive correlation; the salinity increments are associated with the increment of disease incidence (Fig. 9).

Data presented in Fig. (9-A) showed that, soaking cucumber seeds (produced in 2015) in HS'c and cultivating them in different saline conditions have affected the incidence of root rot disease caused by *R. solani*. Where, in the case of lowest tested salinity (2.36), soaking seeds in 0.3% HS'c has significantly reduced the disease incidence to 40 % (45% as disease incidence DI) with reference to control one. While, the foliar application of 100 SA has no significant difference regarding the control. Whereas, the application of 200 SA has reduced the infection percentage up to 27% (DI= 55%) comparing to the control. Finally, the combination of 0.3% HS soaked seeds and 200 SA sprayed seedling has reduced the infection percentage to 73% (DI= 20%) regarding the control with no applications, followed by the combination of 0.3 HS and 100 SA that reduced the DI % up to 53% (DI= 35).

While, soaking seeds in 0.3 HS'c has significantly reduced the DI % in all other salinity levels (4, 5 and 6) up to 12, 11 and 15 % (DI= 75, 85 & 85%, respectively) comparing to control ones (DI= 85, 95 & 100%, respectively). Whereas, SA application separately on plants with both concentration (100 or 200) has significant reduction effect in the two salinity levels 4 and 6 on DI % up to 12 and 15% (DI= 75 and 85%, respectively) comparing to control ones (DI= 85 and 100%, respectively) in case of 100 SA. Where, the DI reduction in the salinity levels of 4, 5 and 6 are up to 24, 16 and 25% (DI= 65, 80 & 75%, respectively) comparing to control ones (DI= 85, 95 & 100%, respectively) for 200 SA (Fig. 9-A).

Generally, all treatments of HS associated with SA have significant effect in DI % reduction (Fig. 9-A). Since, 0.3 HS combined with 100 SA treatments in the ascending soil EC content is associated with DI significant reduction up to 24, 21 and 25% (DI= 65, 75 & 75%, respectively) comparing to control ones (DI= 85, 95 & 100%, respectively). This significant reduction trend of DI % is the same for the associated treatment 0.3 HS and 200 SA in salinity levels of 4, 5 and 6 to be up to 35, 32 and 30% (DI= 55, 65 & 70%, respectively) comparing to control ones (DI= 85, 95 & 100%, respectively) (Fig. 9-A).

The findings for seeds produced in 2016 which infected by *R. solani* fungus are affected in different way comparing to seeds produced in 2015 in the DI reduction percentage (Fig. 9-B). Where, there are significant DI reduction percentage in all tested salinity (2.36, 4, 5 and 6), when soaking seeds in 0.3% HS'c to 40, 12, 11 & 20 % (DI= 45, 75, 85 & 80%, respectively) regarding control ones (DI= 75, 85, 95 & 100%, respectively). While, the 100 SA foliar application has significant DI reduction of 40% (DI= 75) only in salinity level of five regarding control one (DI= 85). Whereas, the application of 200 SA has reduced the infection percentage in lower salinity levels of 2.36 and 4 to 27 & 12% (DI= 55 & 75 respectively) regarding control ones (DI= 75 & 85, respectively). Finally, the combination of 0.3% HS soaked seeds and 200 SA treatments in the soil EC content (2.36, 4, 5 & 6) is associated with DI significant reduction up to 47, 24, 21 and 20% (DI= 40, 65, 75 & 80%, respectively) comparing to control ones (DI= 75, 85, 95 & 100%, respectively). This significant reduction is the same values of DI % for the associated treatment 0.3 HS and 100 SA in salinity levels of 2.36, 4 and 5. While, for the highest tested salinity level, DI significant reduction is 15 % (DI= 85%) regarding control one (DI= 100%) (Fig. 9-B).

For the seeds produced in 2017, there are significant DI reduction percentage in all tested salinity (2.36, 4, 5 and 6), when soaking seeds in 0.3% HS'c to 7, 12, 11 & 20% (DI= 70, 75, 85 & 80%, respectively) regarding control ones (DI= 75, 85, 95 & 100%, respectively). While, the 100 or 200 SA foliar applications have the same significant values of DI reduction percentages at the same salinity levels found in seeds produced 2016. Finally, the combination of 0.3% HS soaked seeds and 200 SA treatments in the soil EC content (2.36, 4, 5 & 6) is associated with DI significant reduction up to 47, 24, 26 and 30% (DI= 40, 65, 70 & 70%, respectively) comparing to control ones (DI= 75, 85, 95 & 100%, respectively). This significant reduction values; of DI % for the associated treatment 0.3 HS and 100 SA in salinity levels of 2.36, 4, 5 & 6; are 13, 24, 26 and 20% (DI= 65, 65, 75 & 80%, respectively) comparing to control ones (DI= 75, 85, 95 & 100%, respectively) (Fig. 9-C).

Data shown in Fig. 9, indicates that the root rot disease incidence caused by *R. solani* could be decreased by using of soaking seeds of 0.3 % HS (with different period/production year) and/or spraying cucumber seedlings.

On the other hand, the disease index or severity (DIx) values caused by *R. solani* infection have significantly affected by different application by SA concentrations (100, 200) and or soaking seed with 0.3 HS'c (Fig. 10).

Data presented in Fig. 10 indicates that the presence of significant DIx reduction percentage in all tested salinity (2.36, 4, 5 and 6). Where soaking seeds in 0.3% HS'c reduces DIx percentage to 40, 17, 33 & 28% (DIx= 155, 245, 235 & 280) regarding control ones (DIx= 260, 295, 350 & 390), respectively for seeds produced 2015 (Fig. 10-A). While, for seeds produced 2016, it reduces DIx % to 39, 12 & 23% (DIx= 160, 260 & 300) under 2.36, 4 and 6 salinity levels regarding control ones (DIx= 260, 295 & 390), respectively. No significant difference in DIx is observed for this treatment under salinity of five regarding the control (Fig. 10-B). Whereas, for seeds produced in 2017, DIx % is reduced to 11, 17, 17 & 21 % (DIx= 245, 245, 290 & 310) under 2.36, 4 and 6 salinity levels regarding control ones (DIx= 275, 295, 350 & 390), respectively (Fig. 10-C).

**Table 4. Influence of soaking in different humic substance concentrations on some germination parameters of stored cucumber (*Cucumis sativus* L.) seeds**

| Treatment | G% | | | | GV | | | | VI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2017 | Mean | 2015 | 2016 | 2017 | Mean | 2015 | 2016 | 2017 | Mean |
| $T_0$ | 60.00 | 96.00 | 96.00 | **84.00** | 29.17 | 76.04 | 102.52 | **69.24** | 185.34 | 543.95 | 513.93 | **414.41** |
| $T_1$ | 42.00 | 100.0 | 96.00 | **79.33** | 26.78 | 106.58 | 113.10 | **82.16** | 137.48 | 390.00 | 499.95 | **342.48** |
| $T_2$ | 46.00 | 90.00 | 98.00 | **78.00** | 20.18 | 92.83 | 115.97 | **76.33** | 47.64 | 824.38 | 437.33 | **436.45** |
| $T_3$ | 58.00 | 90.00 | 98.00 | **82.00** | 37.43 | 101.25 | 106.88 | **81.86** | 185.74 | 846.56 | 642.94 | **558.41** |
| $T_4$ | 96.00 | 64.00 | 98.00 | **86.00** | 89.35 | 35.90 | 117.55 | **80.93** | 563.04 | 272.10 | 629.84 | **488.33** |
| $T_5$ | 52.00 | 94.00 | 98.00 | **81.33** | 24.22 | 93.73 | 117.05 | **78.33** | 140.33 | 963.52 | 725.86 | **609.90** |
| $T_6$ | 36.00 | 88.00 | 98.00 | **74.00** | 20.73 | 99.80 | 115.22 | **78.58** | 82.61 | 291.55 | 578.44 | **317.54** |
| $T_7$ | 44.00 | 92.00 | 100.0 | **78.67** | 22.75 | 89.87 | 117.67 | **76.76** | 109.49 | 955.87 | 357.67 | **474.34** |
| $T_8$ | 24.00 | 92.00 | 98.00 | **71.33** | 11.07 | 91.12 | 117.22 | **73.13** | 20.15 | 959.80 | 699.72 | **559.89** |
| $T_9$ | 44.00 | 90.00 | 94.00 | **76.00** | 23.90 | 82.67 | 108.15 | **71.57** | 68.34 | 875.45 | 396.06 | **446.62** |
| $T_{10}$ | 40.00 | 88.00 | 96.00 | **74.67** | 20.33 | 87.13 | 116.10 | **74.52** | 66.90 | 575.94 | 582.15 | **408.33** |
| $T_{11}$ | 50.00 | 96.00 | 96.00 | **80.67** | 24.58 | 93.85 | 112.52 | **76.98** | 82.29 | 214.03 | 158.40 | **151.57** |
| $T_{12}$ | 54.00 | 92.00 | 96.00 | **80.67** | 24.65 | 96.20 | 103.18 | **74.68** | 125.10 | 382.91 | 361.28 | **289.76** |
| $T_{13}$ | 38.00 | 92.00 | 92.00 | **74.00** | 20.13 | 94.70 | 112.70 | **75.84** | 63.19 | 602.57 | 300.53 | **322.10** |
| $T_{14}$ | 56.00 | 80.00 | 94.00 | **76.67** | 25.02 | 78.00 | 105.82 | **69.61** | 88.00 | 166.68 | 322.16 | **192.28** |
| $T_{15}$ | 56.00 | 86.00 | 94.67 | **78.89** | 24.18 | 84.35 | 106.27 | **71.60** | 90.48 | 147.82 | 250.62 | **162.97** |
| $T_{16}$ | 24.00 | 90.00 | 96.00 | **70.00** | 15.57 | 87.75 | 110.02 | **71.11** | 52.56 | 118.75 | 514.16 | **228.49** |
| $T_{17}$ | 32.00 | 90.00 | 94.00 | **72.00** | 16.22 | 79.58 | 95.15 | **63.65** | 48.22 | 113.10 | 322.02 | **161.11** |
| $T_{18}$ | 46.00 | 76.00 | 98.00 | **73.33** | 24.65 | 82.60 | 107.88 | **71.71** | 82.02 | 254.04 | 320.26 | **218.77** |
| $T_{19}$ | 64.00 | 90.00 | 100.0 | **84.67** | 31.23 | 79.42 | 112.67 | **74.44** | 135.43 | 190.67 | 287.67 | **204.59** |
| $T_{20}$ | 32.00 | 96.00 | 96.00 | **74.67** | 17.87 | 82.93 | 106.60 | **69.13** | 32.02 | 250.35 | 232.21 | **171.52** |
| $T_{21}$ | 38.00 | 82.00 | 96.00 | **72.00** | 18.63 | 60.12 | 101.10 | **59.95** | 24.84 | 82.56 | 142.87 | **83.42** |
| $T_{22}$ | 26.00 | 88.00 | 98.00 | **70.67** | 11.93 | 80.30 | 97.72 | **63.32** | 26.13 | 106.71 | 413.97 | **182.27** |
| $T_{23}$ | 40.00 | 80.00 | 94.00 | **71.33** | 19.50 | 81.00 | 107.15 | **69.22** | 40.67 | 286.61 | 195.45 | **174.24** |
| $T_{24}$ | 38.00 | 96.00 | 98.00 | **77.33** | 18.47 | 95.77 | 100.22 | **71.48** | 20.88 | 521.59 | 198.30 | **246.92** |
| $T_{25}$ | 18.00 | 86.00 | 88.00 | **64.00** | 5.55 | 86.52 | 98.80 | **63.62** | 3.13 | 264.79 | 155.96 | **141.29** |
| **Mean** | **44.38c** | **88.62b** | **96.18a** | | **24.00c** | **85.39b** | **108.66a** | | **97.00c** | **430.86a** | **393.84b** | |
| **$LSD_{0.05}$** | **Y** | **T** | **Y x T** | | **Y** | **T** | **Y x T** | | **Y** | **T** | **Y x T** | |
| | **2.85**** | **5.35**** | **9.27**** | | **2.84**** | **2.96**** | **5.13**** | | **56.35**** | **54.85**** | **95.01**** | |

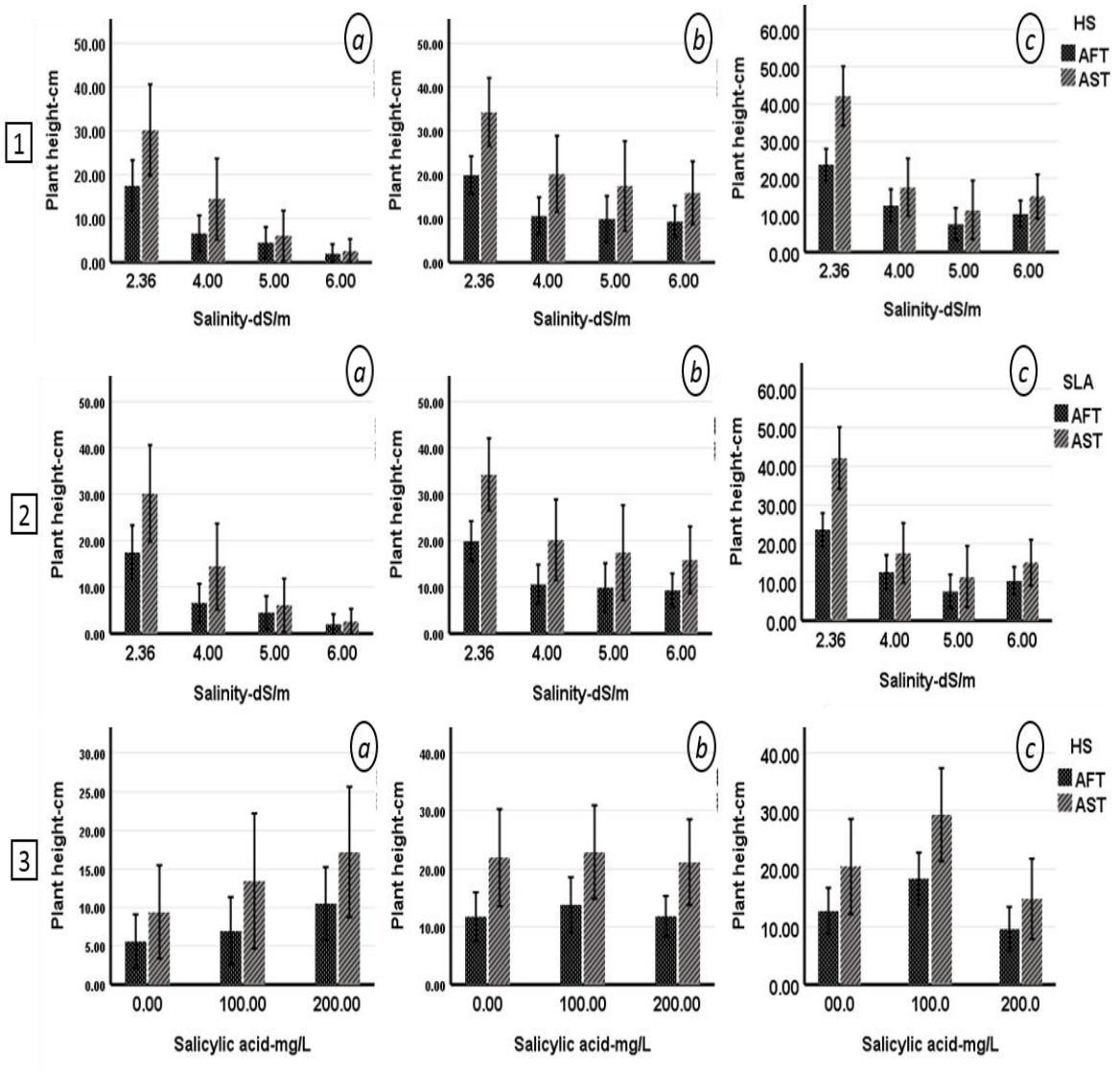
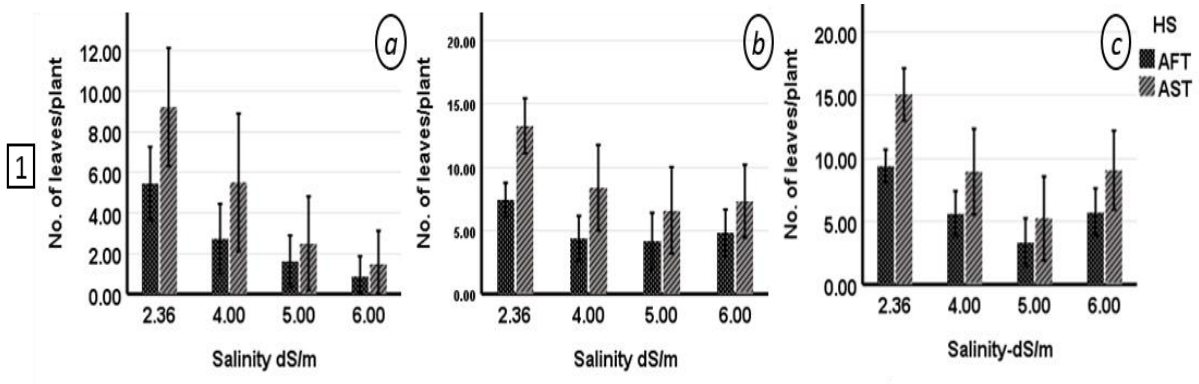



**Fig. 6. Interaction Influence of humic substances soaking and different salinity levels on cucumber plant height produced in a: 2015, b: 2016, c: 2017, where, 1: humic substances (HS) and salinity interaction effect, 2: salicylic acid (SLA) and salinity interaction effect, and 3: humic substances (HS) and salicylic acid (SLA) interaction effect**



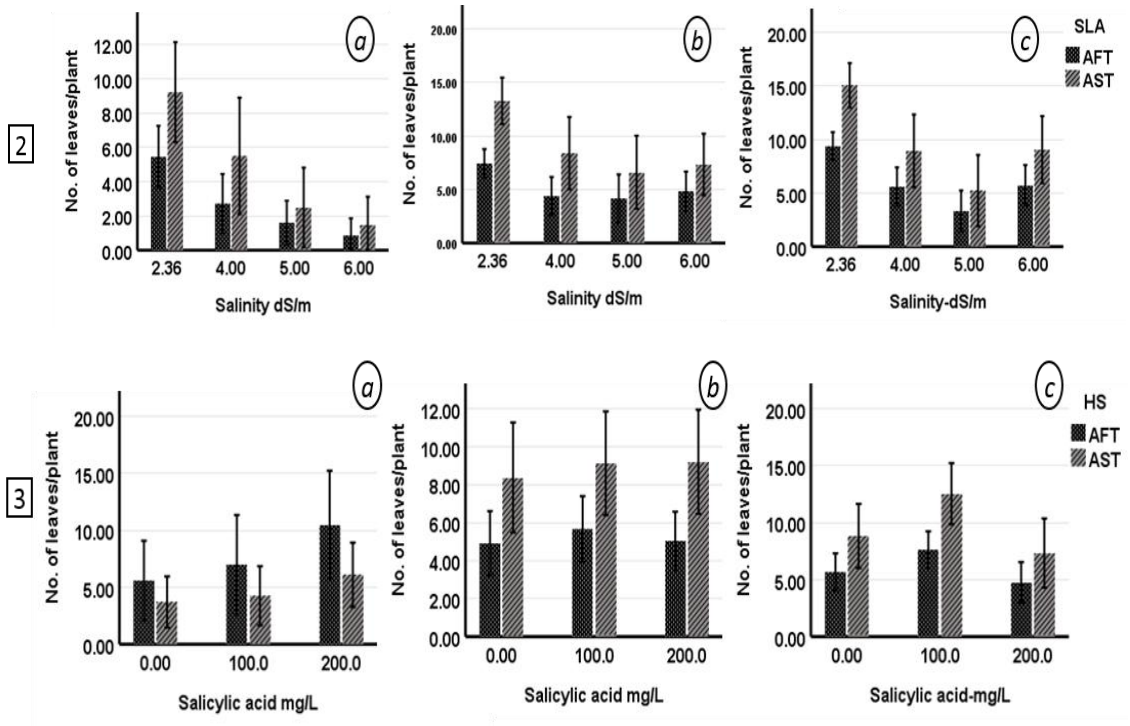
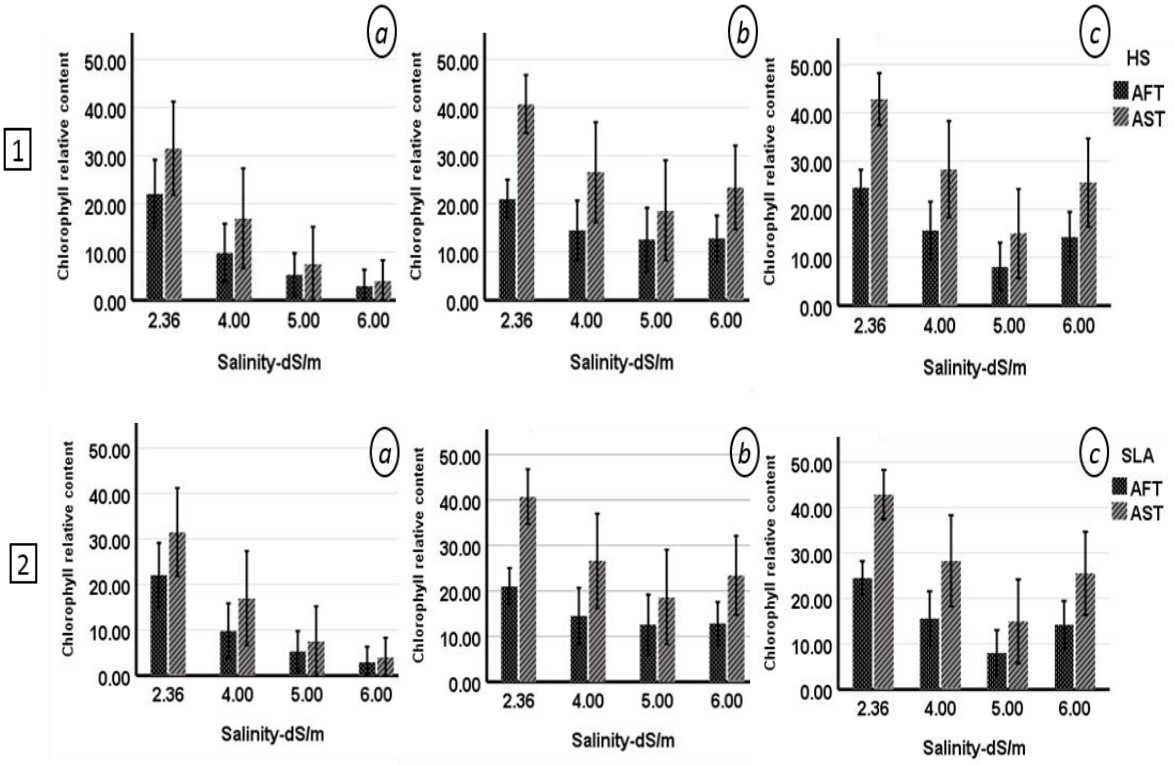



**Fig. 7. Interaction Influence of humic substances soaking and different salinity levels on cucumber number of leaves/plant produced in a: 2015, b: 2016, c: 2017, where, humic substances (HS) and salinity interaction effect, 2: salicylic acid (SLA) and salinity interaction effect, and 3: humic substances (HS) and salicylic acid (SLA) interaction effect**



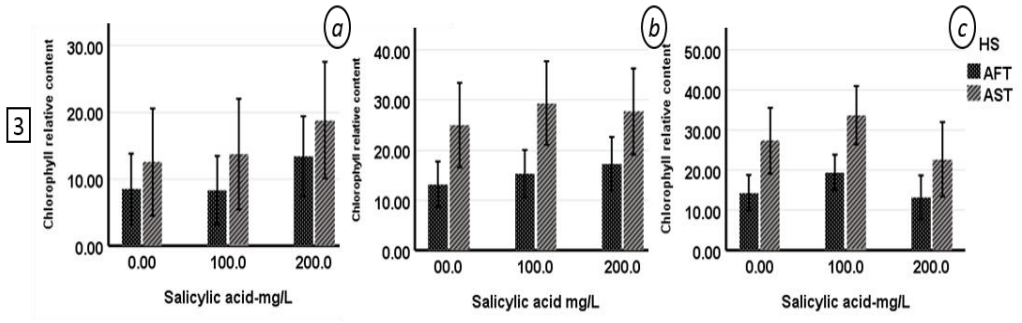



**Fig. 8. Interaction Influence of humic substances soaking and different salinity levels on cucumber chlorophyll relative content produced in a: 20 15, b: 2016, c: 2017, where, humic substances (HS) and salinity interaction effect, 2: salicylic acid (SLA) and salinity interaction effect, and 3: humic substances (HS) and salicylic acid (SLA) interaction effect**

The 100 SA foliar applications for 2015-seeds under salinity conditions; 4, 5 & 6 have significant DIx reduction % as follow: 10, 7 & 19% (DIx= 265, 325 & 315) regarding control ones (DIx= 295, 350 & 390), respectively. Whereas, this treatment has no significant difference in the lowest salinity content (2.36) (Fig. 10-A). For 2016-seeds, 100 SA foliar applications have significant DIx reduction % as follow: 13, 10, 9 & 8 % (DIx=225, 265, 320 &360) regarding control ones (DIx= 260, 295, 350 & 390), respectively under tested salinity conditions in an ascending order (Fig. 10-B). The DIx of 2017-seeds is reduced significantly as follow: 20, 10, 5 & 13% (DIx=220, 265, 335 &340) regarding control ones (DIx= 275, 295, 350 & 390), respectively under different salinity conditions in an ascending order (Fig. 10-C).

The separate application of 200 SA has significantly reduced the infection percentage for 2015-seeds all salinity levels in ascending order. Which reduces DIx % to 13, 20, 16 & 28 % (DIx= 225, 235, 295 & 280), respectively regarding control ones (Fig.10-A). DIx reduction percentage for 2016-seeds are 25, 20, 16 & 24 % (DIx= 195, 235, 295 & 295), respectively regarding control ones (Fig. 10-B). Meanwhile, DIx was reduced for 2017-seeds to 20, 10, 5 & 13% (DIx=220, 265, 335 & 340), respectively regarding control ones under different salinity conditions in an ascending order (Fig. 10-C).

Finally, the combination of 0.3% HS soaked seeds and 200 SA treatments in the soil EC content (2.36, 4, 5 & 6) for 2015-seeds is associated with DIx significant reduction up to 65, 32, 33 and 37% (DIx= 90, 200, 235 & 245), respectively comparing to control ones (Fig. 10-A). For 2016-seeds, DIx values are significantly reduced up to 50, 32, 30 and 32% (DIx= 130, 200, 245 & 265), respectively comparing to control ones (Fig. 10-B). While, for 2017-seeds, the DIx values are significantly reduced up to 50, 39, 30 and 33% (DIx= 130, 180, 245 & 260), respectively comparing to control ones (Fig. 10-C). Followed by the combination of 0.3% HS soaked seeds and 100 SA treatments in the soil EC content (2.36, 4, 5 & 6) is associated with DIx significant reduction up to 29, 27, 21 and 28% (DIx= 185, 215, 275 & 280), respectively regarding control ones (Fig. 10-A) for 2015-seed. While, for 2016-seeds, DIx significant reduction is observed up to 52, 27, 19 and 23% (DIx= 130, 215, 285 & 300), respectively regarding control ones (Fig. 10-B). Where, for 2017-seeds,under 2.36, 4 and 6 salinity conditions DIx is significantly reduced up to 20, 12and 13% (DIx= 220, 265 & 340) comparing to control ones (DIx= 275, 295 & 390), respectively. No significant difference is observed for DIx, in case of the treatment of 100 SA and salinity of five (Fig. 10- C).

**b) Disease incidence of *F. solani***

Cucumber root rot disease incidence caused by *F. solani* (Fig. 11) differs from that caused by *R. solani* (Fig. 9), where it is extremely high that combined with *F. solani*. In addition to the presence of a positive correlation between the salinity and *F. solani* disease incidence (Fig. 11). Data presented in Fig. 11-A for 2015-seeds showed that, low effect of the different treatments HS and SA in decrement of the DI. Where, soaking cucumber seeds in HS'c under different saline conditions have affected the DI reduction percentage in a range of 5% (Salinity 2.36) and 10% for other salinity content. Whereas, for 2016-seeds, DI reduction % are 40, 17, 11 and 20% (DI= 45, 70, 85 & 80%), respectively comparing to control ones in ascending order of the salinity tested levels (Fig. 11-B). for 2017-seeds, DI reduction % are 12, 21 and 15 % (DI=

75, 75 & 85%) under salinity of 2.36, 4 and 6, respectively. No significant reduction is observed in the five-salinity level (Fig. 11-C).

The 100 SA foliar applications for 2015-seeds under 4-salinity condition has DI reduction percentage of 10%, where under other salinity condition has significantly reduced DI % to 15% (Fig. 11-A). For the 2016-seeds, no significant reduction for the DI % has observed (Fig. 11-B). Whereas, for 2017-seeds only the 100 SA spraying reduction effect on DI % is found in the lower salinity levels (2.36 and 4) are 12 and 21% (Fig. 11-C). The 200 SA foliar applications for 2015-seeds under tested salinity conditions have DI reduction percentage to 16, 20, 25& 25% (Fig. 11-A). For the 2016-seeds, only the 200 SA spraying reduction effect on DI % is found in the lower salinity levels (2.36 and 4) are 12 and 21% (Fig. 11-B). Whereas, for 2017-seeds, the DI % has reduced to 41, 21, 16 & 15% under tested salinity conditions in ascending matter (Fig. 11-C).

Finally, the combination of 0.3% HS soaked seeds and 200 SA treatments in the soil EC content (2.36, 4, 5 & 6) for 2015-seeds is associated with DI significant reduction up to 27, 30, 25 and 25%, respectively comparing to control ones (Fig. 11-A). For 2016-seeds, DI values are significantly reduced up to 47, 29, 32 and 25%, respectively comparing to control ones (Fig. 11-B). While, for 2017-seeds, the DI values are significantly reduced up to 53, 32, 26 and 30%, respectively compares to control ones (Fig. 11-C). whereas, the combination of 0.3% HS soaked seeds and 100 SA treatments in the soil EC content (2.36, 4, 5 & 6) for 2015-seeds is associated with DI significant reduction up to 16, 25, 20 and 15%, respectively comparing to control ones (Fig. 11-A). For 2016-seeds, DI values are significantly reduced up to 47, 29, 21 and 20%, respectively comparing to control ones (Fig. 11-B). While, for 2017-seeds, the DI values are significantly reduced up to 29, 27, 16 and 20%, respectively is comparing to control ones (Fig. 11-C).

On the other hand, the DIx is affected by the salinity increment. The combined treatment of 200 SA and HS showed high significant reduction for the *F. solani* DI percentage in six-salinity levels (Fig. 11) in all the three seed production year.

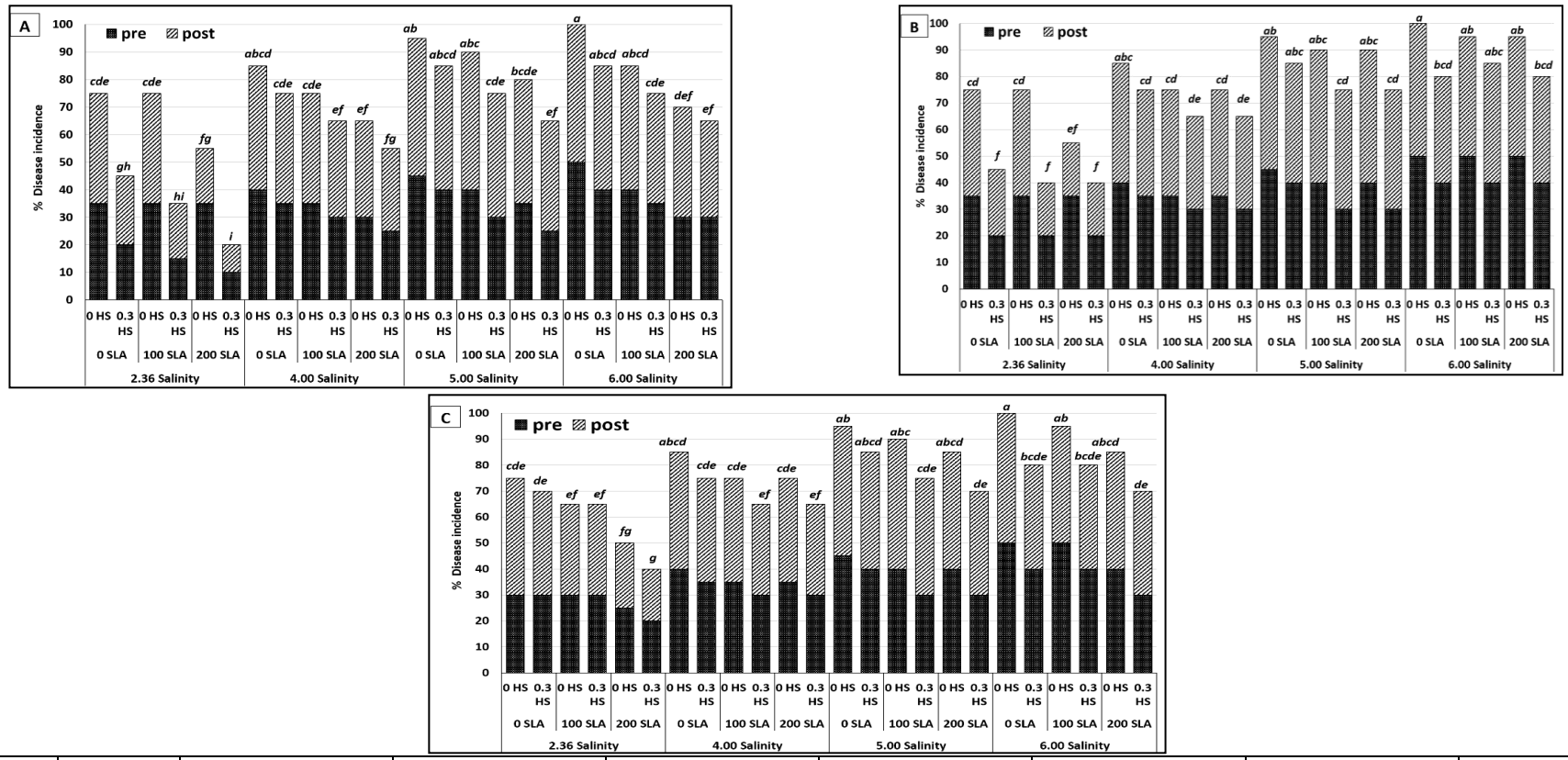
The disease index or severity (DIx) values caused by *F. solani* infection have significantly affected by different application by SA concentrations (100, 200) and or soaking seed with 0.3 HS'c (Fig. 12).

The treatments of 0.3% HS and 200 SA under soil EC content levels (2.36, 4, 5 & 6) is associated with DIx significant reduction for 2015-seeds up to 40, 26, 42 and 29 %, respectively comparing to control ones (Fig. 12-A). Where, for 2016-seeds, DIx values are significantly reduced up to 50, 24, 22 and 33 %, respectively comparing to control ones (Fig. 12-B). While, for 2017-seeds, the DIx values are significantly reduced up to 57, 45, 30 and 34%, respectively comparing to control ones (Fig. 12-C). Followed by the treatments of 0.3% HS combined with 100 SA treatments in the soil EC content (2.36, 4, 5 & 6) is associated with DIx significant reduction up to 24, 26, 25 and 30%, respectively regarding control ones (Fig. 12-A) for 2015-seed. While, for 2016-seeds, DIx significant reduction is observed up to 50, 35, 21 and 25%, respectively regarding control ones (Fig. 12-B). Where, for 2017-seeds, DIx is significantly reduced up to 57, 12 45, 30 and 34 % comparing to control ones, respectively. (Fig. 12-C).

Data obtained for *R. solani* mentioned above (Fig. 10) are similar to the obtained for *F. solani* (Fig. 12). Which, indicate that the presence of significant DIx reduction percentage in all tested salinity (2.36, 4, 5 and 6) and the other SA and Hs treatments.

From the data mentioned above, different [38,39] had illustrated the different functional actions of HS, their ability to improve plant growth in diverse plant species and growth conditions. Whereas, Chen et al. [40] proposed that HS promote plant growth by improving bioavailability of certain nutrients in soil, principally iron and zinc. Nardi et al. [41] suggested that the direct effect of HS on plant metabolism. [39] reported that the root application of a purified humic acid causes a significant increase in shoot growth that is associated with an enhancement in root $H^{+}$ - ATPase activity, an increase in nitrate shoot concentration, and a decrease in roots.

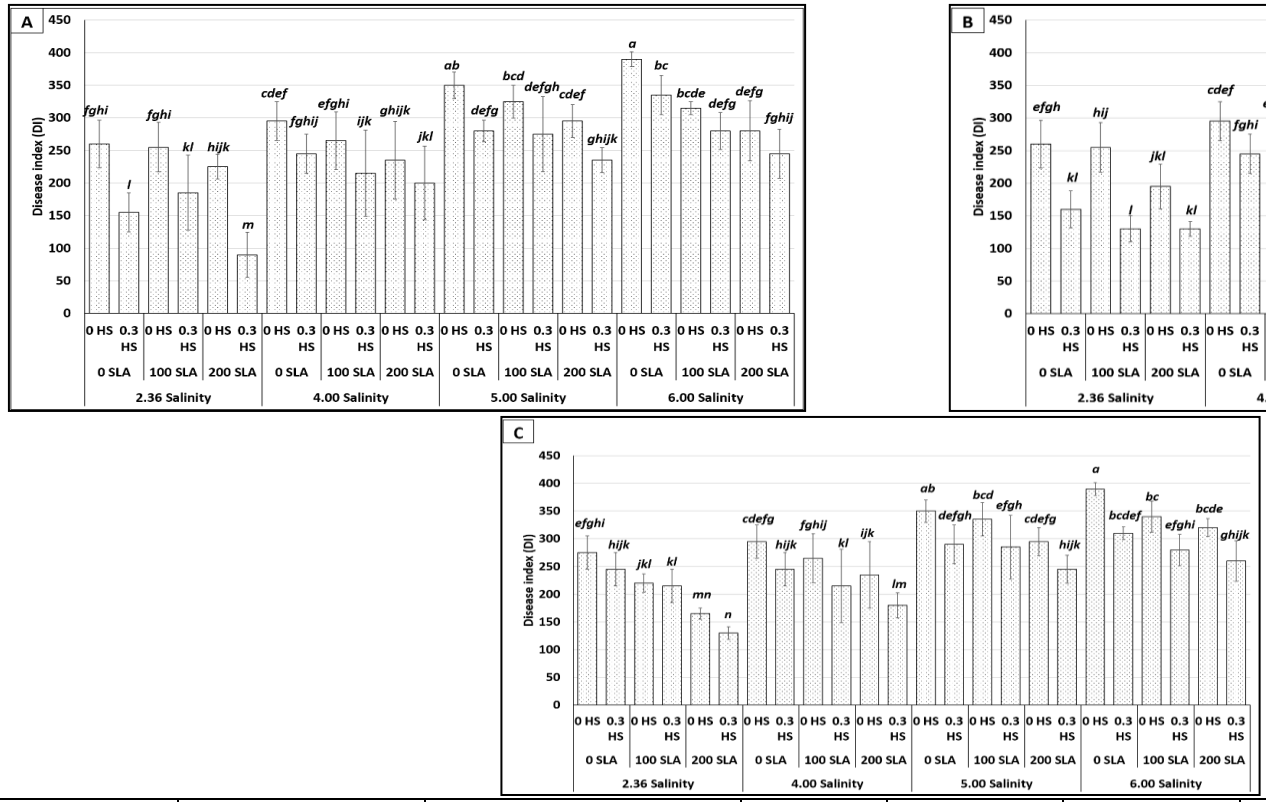
Application of HS'c alone or/and SA have affected the cucumber seedling growth parameters (plant height, number of leaves and chlorophyll content. The results obtained agrees with El-Mohamedy et al. [42] findings, who reported the effect of salicylic acid application controlling tomato root rot caused by *R. solani, F. solani* and *Sclerotium rolfsii* as plant chemical resistance inducers.

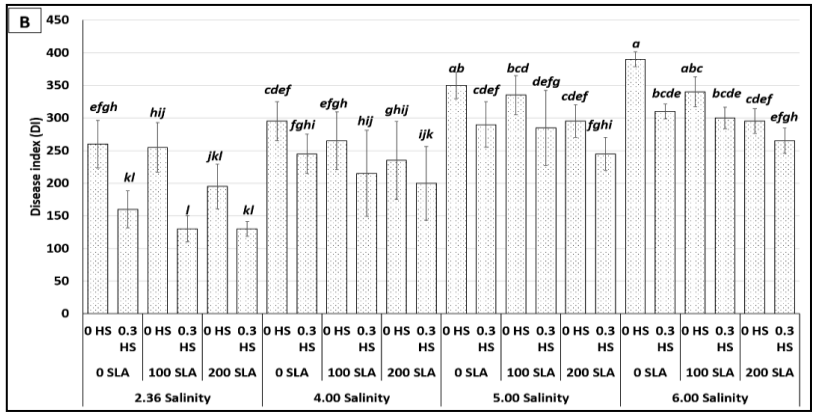



| DI% LSD | | humic acid (HA) | SA | Salinity | SA * Salinity | HA * Salinity | HA *SA | HA*SA*Salinity |
|---|---|---|---|---|---|---|---|---|
| | 2015 | 4.72305 | 5.78453 | 6.67940 | 11.56905 | 9.44609 | 8.18056 | 16.36111 |
| | 2016 | 4.88265 | 5.98000 | 6.90511 | 11.96001 | 9.76531 | 8.46805 | 17.00771 |
| | 2017 | 4.72305 | 5.78453 | 6.67940 | 11.56905 | 9.44609 | 8.18056 | 16.36111 |

**Fig. 9. Influence of humic acid soaking solutions (0 and 0.3%), salinity levels (2.36, 4, 5 and 6 ds/m) and Salicylic acid spraying (0, 100 and 200 mg/L) on cucumber root rot disease incidence caused by *Rhizoctonia solani* (pre- and post- emerging root rot) of seeds and seedlings under greenhouse condition A: seeds produced 2015; B: seeds produced 2016; C: seeds produced 2017**

*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test*



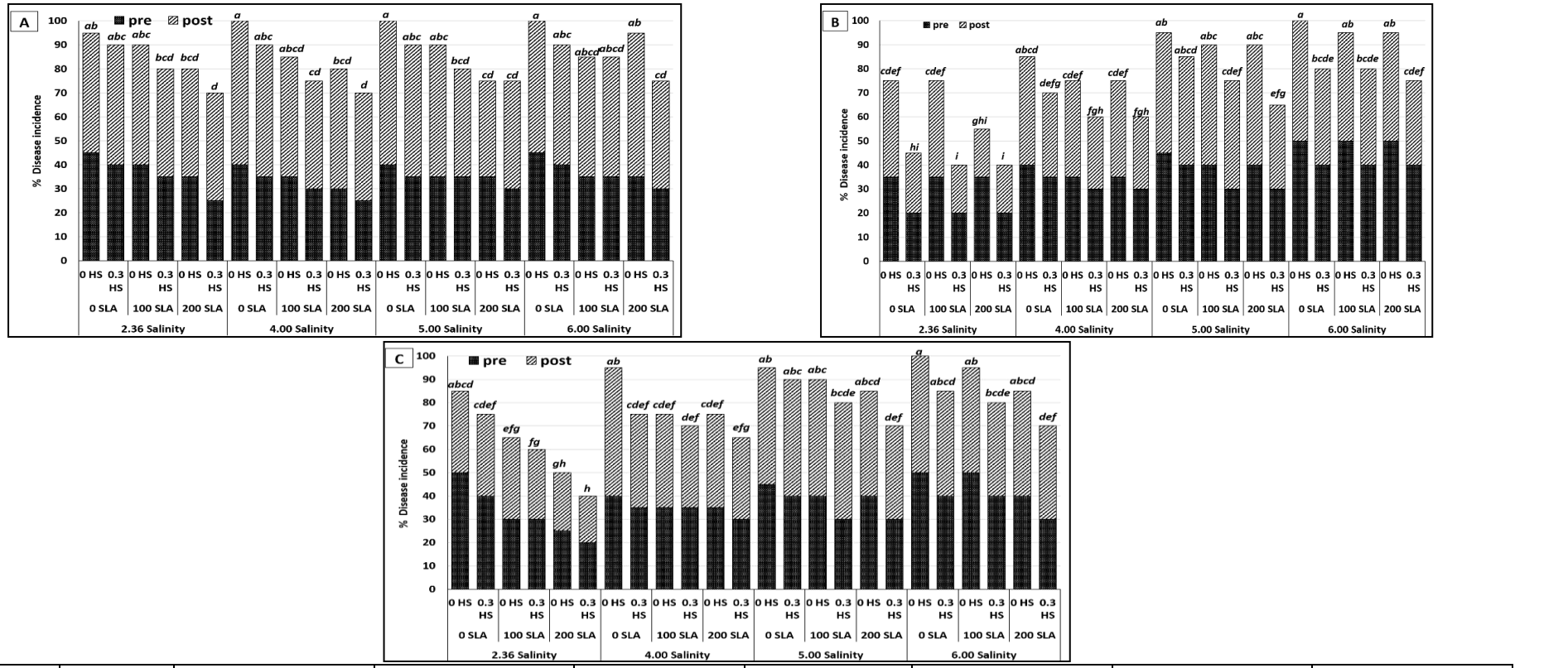




| LSD | | humic acid (HA) | SA | Salinity | SA * Salinity | HA * Salinity | HA *SA | HA*SA*Salinity |
|---|---|---|---|---|---|---|---|---|
| | 2015 | 15.43534 | 18.90435 | 21.82887 | 37.80871 | 30.87068 | 26.73479 | 53.46959 |
| | 2016 | 15.50422 | 18.98871 | 21.92628 | 37.97742 | 31.00844 | 26.88917 | 54.00574 |
| | 2017 | 13.38459 | 16.39271 | 18.92867 | 32.78541 | 26.76918 | 23.18279 | 46.36558 |

**Fig. 10. Influence of humic acid soaking solutions (0 and 0.3%), salinity levels (2.36, 4, 5 and 6 ds/m) and Salicylic acid spraying (0, 100 and 200 mg/L) on cucumber root rot disease index % caused by *Rhizoctonia solani* of seeds and seedlings under greenhouse condition A: seeds produced 2015; B: seeds produced 2016; C: seeds produced 2015**

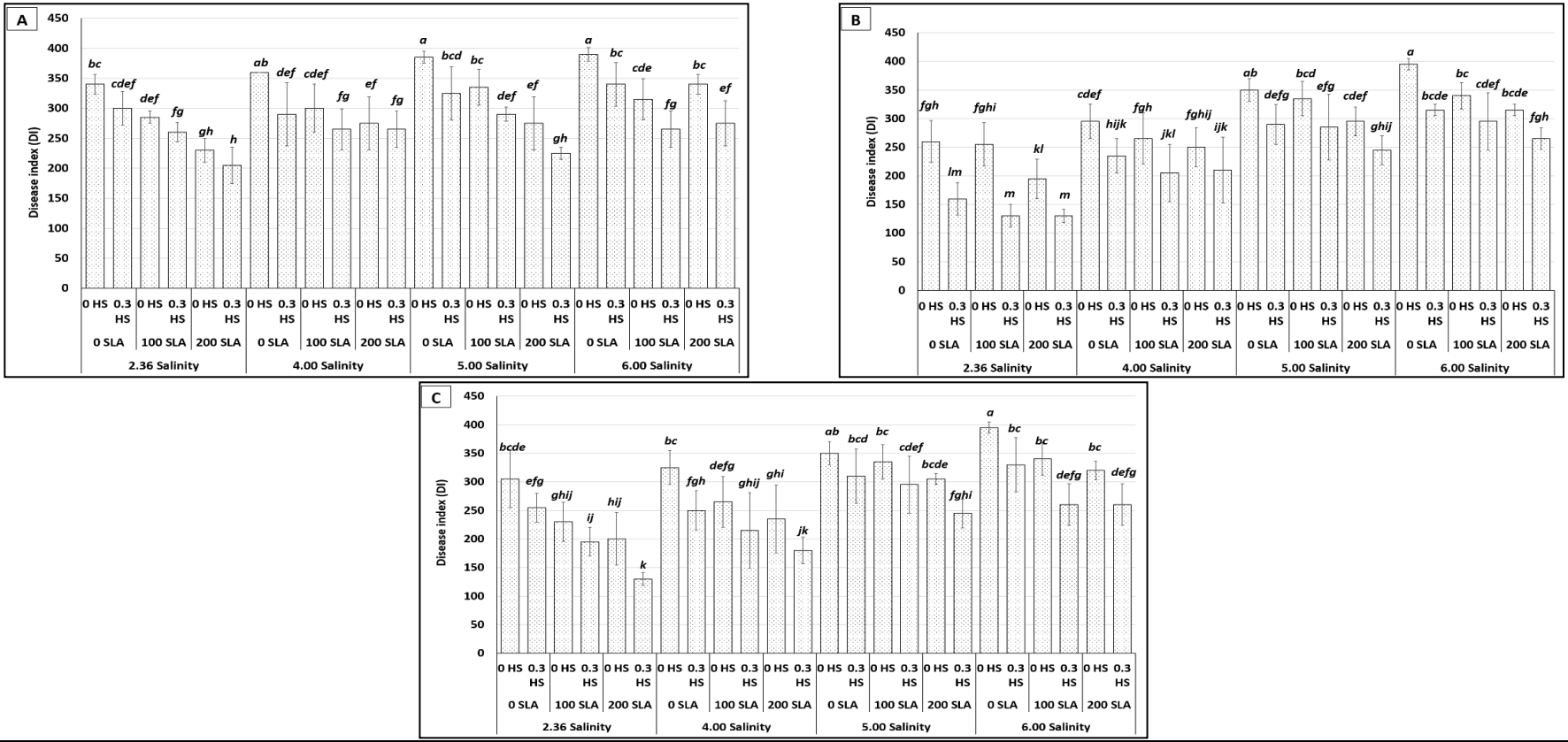
*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test*




| DI% LSD | | humic acid (HA) | SA | Salinity | SA * Salinity | HA * Salinity | HA *SA | HA*SA*Salinity |
|---|---|---|---|---|---|---|---|---|
| | 2015 | 4.96053 | 6.07539 | 7.01525 | 12.15077 | 9.92106 | 8.59189 | 17.18379 |
| | 2016 | 4.64944 | 5.69437 | 6.57530 | 11.38875 | 9.29887 | 8.05306 | 16.10612 |
| | 2017 | 5.05240 | 6.18790 | 7.14517 | 12.37580 | 10.10480 | 8.75102 | 17.50203 |

**Fig. 11. Influence of humic acid soaking solutions (0 and 0.3%), salinity levels (2.36, 4, 5 and 6 ds/m) and Salicylic acid spraying (0, 100 and 200 mg/L) on cucumber root rot disease incidence caused by *Fusarium solani* (pre- and post- emerging root rot) of seeds and seedlings under greenhouse condition A: seeds produced 2015; B: seeds produced 2016; C: seeds produced 2017**

*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test*




| LSD | | humic acid (HA) | SA | Salinity | SA * Salinity | HA * Salinity | HA *SA | HA*SA*Salinity |
|---|---|---|---|---|---|---|---|---|
| | 2015 | 12.9800 | 14.93943 | 17.25057 | 29.87886 | 24.39599 | 21.12755 | 42.25509 |
| | 2016 | 13.60612 | 16.66402 | 19.24196 | 33.32805 | 27.21224 | 23.56649 | 47.13298 |
| | 2017 | 15.01237 | 18.38633 | 21.23070 | 36.77265 | 30.02475 | 26.00219 | 52.00439 |

**Fig. 12. Influence of humic acid soaking solutions (0 and 0.3%), salinity levels (2.36, 4, 5 and 6 ds/m) and Salicylic acid spraying (0, 100 and 200 mg/L) on cucumber root rot disease index % caused by *Fusarium solani* of seeds and seedlings under greenhouse condition A: seeds produced 2015; B: seeds produced 2016; C: seeds produced 2015**

*Values followed by the same letters are not significantly different at P=0.05 according to Fisher's LSD test*

## 4. CONCLUSION

The combined treatment; soaking seeds in 0.3% HS (for different period/year) and spraying seedlings with 200 SA had significantly reduced the disease incidence (DI) and disease index of both *R. solani* and *F. solani* specially in the lowest and highest salinity conditions.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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