

## Biological and chemical control of sunflower basal stem rot caused by *Sclerotium rolfsii*

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Received on: 15-5-2021

Accepted on: 24-7-2021

### ABSTRACT

The studies showed significant inhibitory effects of potassium bicarbonate ( $\text{KHCO}_3$ ) either on growth parameters of *Sclerotium rolfsii* or in sunflower root/collar. Inhibitory effects were increased by enhancing  $\text{KHCO}_3$  concentration.  $\text{KHCO}_3$  gave significant inhibitory effects against *Sclerotium rolfsii* caused 76.6, 57.69 and 95.78% inhibition for linear growth, sclerotial formation and mycelial dry weight, respectively at 0.6g/l  $\text{KHCO}_3$ .  $\text{KHCO}_3$  provided synergistic effects with *Trichoderma harzianum*, Since the antagonistic potentiality of *T.harzianum* against *S. rolfsii* was increased with increasing  $\text{KHCO}_3$  concentrations.  $\text{KHCO}_3$  caused 80 and 76% inhibition for linear growth and sclerotial formation, respectively. However, the most effective to prevent growth parameters and infectivity of *S.rolfsii* the combinations of potassium bicarbonate and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) with *Trihodermaharzianum* 86.25% and 97.6% inhibition for radial growth and sclerotial formation at  $\text{H}_2\text{O}_2$  (1.2 g/l) + $\text{KHCO}_3$  (0.6 g/l) with *T.harzianum*, respectively. The treatments  $\text{H}_2\text{O}_2$  (1.2 g/l) + $\text{KHCO}_3$  (0.3g/l) with *T.harzianum* and  $\text{H}_2\text{O}_2$  (1.2 g/l) + $\text{KHCO}_3$  (0.6 g/l) with *T.harzianum* achieved 75% and 71% protection against sunflower basal stem rot, respectively. .

**KEYWORDS:** Potassium bicarbonate, *Sclerotium rolfsii*, sunflower, *Trichoderma harzianum* and Hydrogen peroxide .

### 1. INTRODUCTION

Sunflower root rot/ collar rot pathogen *Sclerotium rolfsii* Sacc., teleomorph, *Athelia rolfsii* (Okabe and Matsumoto, 2003). It primarily attacks almost all plant organs including stems, roots, fruits, petioles and leaves under favorable conditions. It commonly occurs in various warm temperature regions of the world, as well as different plant hosts more than 500 plant species (Rajeev and Mukhopadhyay, 2002 and Fu *et al.*, 2002).

Oxidative burst, mediated by  $\text{H}_2\text{O}_2$ , has been recognized as a key component of plant defense response during an incompatible interaction. Elevated levels of  $\text{H}_2\text{O}_2$  also activated the expression of several defense genes to both bacterial and fungal pathogens (Kachroo *et al.*, 2003).  $\text{H}_2\text{O}_2$  is reported to inhibit biotrophic but benefit necrotrophic pathogens. Infection by necrotrophs can result in a massive accumulation of  $\text{H}_2\text{O}_2$  in hosts. The hemibiotroph, *Septoria tritici*, infecting wheat is inhibited by  $\text{H}_2\text{O}_2$  during the biotrophic phase, but a large  $\text{H}_2\text{O}_2$  accumulation occurs in the host during reproduction (Shetty *et al.*, 2007). *Trichoderma spp.* are agriculturally important for their beneficial effects on plant growth and development and for their capability

to induce plant defense responses against pathogens (Harman *et al.*, 2004; Waghunde *et al.*, 2016 and EL-Ashmony *et al.*, 2017). *Trichoderma* are being tested as alternatives to chemical fungicides. (El-Mougy *et al.*, 2012 and Guzman-Valle *et al.*, 2014). There has been considerable interest in the use of baking soda (sodium bicarbonate, ( $\text{NaHCO}_3$ )) and potassium bicarbonate ( $\text{KHCO}_3$ ) for controlling various plant fungal diseases (Smilanick *et al.*, 2006). Bicarbonates are widely used in the food industry (Lindsay, 1985) were found to suppress several fungal diseases of cucumber plants (Ziv and Zitter, 1992). Baking soda sprays provided good control of several plant diseases (Horst *et al.*, 1992; Arimoto *et al.*, 1997; Palmer *et al.*, 1997; Janisiewicz, and Peterson. 2005). Also potassium bicarbonate provided the best protection against plant diseases (Smilanick and Margosan, 1999; Fallik *et al.*, 1996 and Smilanick, *et al.*, 2006). Potassium bicarbonates combined with oil were effective in the control plant diseases (Horst *et al.*, 1992 and Ziv and Zitter and 1992 Abd-El-Kareem *et al.*, 2012). Mixing  $\text{KHCO}_3$  with  $\text{H}_2\text{O}_2$  was effective to control okra powdery mildew (Tantawy *et al.*, 2020)

The present study was planned to explore the effects of  $\text{H}_2\text{O}_2$ +potassium bicarbonate mixture and/or

bioagent *T.harzianum* on growth parameters of *S.rolfsii* and on its infectivity toward sunflower plants.

## 2. MATERIALS AND METHODS

### 2.1. Causal agent and bioagent:

The most aggressive *Sclerotium rolfsii* isolate Sr1 which was isolated from sunflower basal stem rot and the bioagent *Trichoderma harzianum* isolate No. A1 d (TrA1d) were used throughout this study (EL-Ashmony *et al.*, 2017).

### 2.2. Preparation of test solutions:

Stock solutions of three mixtures of potassium bicarbonate ( $\text{KHCO}_3$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were prepared with distilled water. Different concentrations of  $\text{KHCO}_3 + \text{H}_2\text{O}_2$  mixtures, i.e. (0.15 g/l  $\text{KHCO}_3 + 1.2$  g/l  $\text{H}_2\text{O}_2$ , 0.3 g/l  $\text{KHCO}_3 + 1.2$  g/l  $\text{H}_2\text{O}_2$  and 0.6 g/l  $\text{KHCO}_3 + 1.2$  g/l  $\text{H}_2\text{O}_2$ ).

### 2.3. Effect of $\text{KHCO}_3$ on some growth parameters of *Sclerotium rolfsii* and *T. harzianum*

$\text{KHCO}_3$  was tested at 0.15, 0.3 and 0.6 g/l concentrations, each was added to autoclaved nutrient agar medium in conical flasks and then dispensed to Petri dishes (15ml medium/plate) and left to solidify. Plates were then inoculated with *S.rolfsii* by placing cork borer made agar disks (5mm) taken from the periphery of fungal colonies grown for 7 days after incubation at 27°C. *Sclerotium rolfsii* were placed in center of Petri plates containing different concentrations of  $\text{KHCO}_3$ .

Plates containing nutrient agar medium without  $\text{KHCO}_3$  were inoculated similarly to be taken for comparison. Data were recorded by measuring the diameter of the fungal growth (two diameters) and their means where the mycelia growth of any dish reached the edge of the plate. When the sclerotia covered the control plates, after 14 days, sclerotia from each plate were harvested, and their numbers were recorded (EL-Bana *et al.*, 2006). As for *T. harzianum* sporulation, the number of spores was calculated from 7 day old cultures by gently brushing spores from the surface of the plate with a fine paintbrush into 5-10 ml distilled water containing 0.05% tween. Each suspension was stirred vigorously by vortex for 1 min to break up spore aggregates. For each suspension, the number of spores was counted using haemocytometer (Morris and Nicholls, 1978).

### 2.4. Effect of $\text{KHCO}_3$ on the mycelial dry weight of *Sclerotium rolfsii* and *Trichoderma harzianum*:

The toxicity of potassium bicarbonate, with different concentrations on growth of *S. rolfsii* or *T. harzianum* isolate in liquid nutrient broth medium (NB) was studied. Equal disks (10 mm in diameter) were taken from actively edge of 7 days old cultures of the tested *S. rolfsii* isolate grown on nutrient agar medium at 27°C and used for inoculation of 250 ml Erlenmeyer flasks containing 50 ml sterilized nutrient medium amended with tested concentration of  $\text{KHCO}_3$ . Concentrations were prepared in sterile distilled water and aliquots were pipetted to NB medium to obtain final concentration of 0.15, 0.3 and 0.6 g/l potassium bicarbonate. For control treatment, conical flasks containing medium without the tested chemical were inoculated similarly to be taken for comparison. Each treatment was incubated at 27°C for 7 days. Mycelial dry weight (MDW) of different treatments was measured (mg MDW per 100 ml liquid medium) after separation of fungal mass by filtration through Whatman No-1 filter paper and dried at 60°C for 48 hrs. The following equation was used to calculate percentage inhibition of fungal growth. (Sutton and Starzyk, 1972).

$$\text{Inhibition\%} = \frac{(\text{MDW of the control} - \text{MDW of the treatment})}{\text{MDW of the control}} \times 100$$

### 2.5. Interaction effects of potassium bicarbonate ( $\text{KHCO}_3$ ) and *Trichoderma harzianum* on *Sclerotium rolfsii* growth in vitro:

$\text{KHCO}_3$  was tested at 0.15, 0.3 and 0.6 g/l concentrations, each was added to autoclaved nutrient agar medium in conical flasks and then dispensed to Petri dishes (15ml medium/plate) and left to solidify. Plates were then inoculated with *S.rolfsii* by placing cork borer made agar disks (10 mm) taken from the periphery of fungal colonies grown for 7 days after incubation at 27°C. The mycelial disks of 10 mm diameter of *Trichoderma harzianum* strain No. A1 d and *Sclerotium rolfsii* were placed opposite to each other on Petri plates containing different concentrations of  $\text{KHCO}_3$ . Plates containing nutrient agar medium without the tested chemical ( $\text{KHCO}_3$ ) were inoculated similarly to be taken for comparison. The data were recorded regularly on the growth of the pathogen and bioagent isolate No. A1 d. Percentage of mycelial growth inhibition (MGI%) was calculated according to the formula  $\text{MGI\%} = \frac{(dc - dt)}{dc} \times 100$

Where, dc= fungal colony diameter in control sets, dt= fungal colony diameter in treatment sets.

## 2.6. Interaction effects of potassium bicarbonate (KHCO<sub>3</sub>), H<sub>2</sub>O<sub>2</sub> and *Trichoderma harzianum* on *Sclerotium rolfsii* growth in vitro:

H<sub>2</sub>O<sub>2</sub> was tested at concentration 0.8 and 1.2 g/l and KHCO<sub>3</sub> was tested at 0.3 and 0.6 g/l each were added to autoclaved nutrient agar medium in conical flasks and then dispensed to Petri dishes (15 ml medium/plate) and left to solidify. Plates were then inoculated with the desired isolate (Sr<sub>1</sub>) of *Sclerotium rolfsii* by placing agar disks (10 mm) taken from the periphery of fungal colonies grown 7 days after incubation at 27°C. Plates containing nutrient agar medium without test chemicals were inoculated similarly to be taken for comparison (Abdou and Galal, 1997). Three plates for each treatment were used, and then incubated at 27°C. Data were recorded as mentioned above. When the sclerotia covered any plate of treated or untreated, after 14 days, sclerotia from each plate were harvested, and their numbers were recorded (EL-Bana *et al.*, 2006).

## 2.7. In vivo interaction effects of H<sub>2</sub>O<sub>2</sub>, KHCO<sub>3</sub>, *T.harzianum* and their combinations (soil drenching) on *S. rolfsii* infectivity to sunflower plants:

The present study was carried out in a greenhouse located at Plant Pathol. Dept., Fac. Agric., Minia Univ., EL-Minia, Egypt.

Effect of bioagent *T.harzianum* and/or H<sub>2</sub>O<sub>2</sub> at concentrations of 0.8 and 1.2 g/l and KHCO<sub>3</sub> at concentration 0.15, 0.3 and 0.6 g/l on resistance of sunflower cv. Sagha 53 to root/collar rot was studied. Hundred seeds for each treatment were wetted by 5ml of each tested treatments for 12 hours overnight while seeds wetted by 5ml distilled water were used for control then treated seeds were coated by *Trichoderma harzianum* solution plus 1 ml tween 20 five hours before planting then seeds were planted in five replicates (4 pot replicates, 5 seed per each pot). Soil was artificially infested with *S. rolfsii* grown in barely grains for 10 days and mixed with soil at 2.5% w/w and transferred into 15 cm diam. Pots, 5 kg soil per pot. Sterilized soil not infested with *S. rolfsii* served as control. Seeds of sunflower were seeded in separate sets, 5 seeds per pot. Soil moisture was adjusted to 50% water hold capacity (Keen and Raczkowski, 1921) and amount of water loss was restored after each 24 hrs.

## 2.8. Disease assessment:

The arbitrary (0-5) disease scale described by (Abd Elrazek *et al.*, 1974) was used to measure the disease severity in which. 0= noinfection, 1= 1- 20 % infection; 2 =21-40 % infection; 3= 41-60% infection; 4 = 61-80 % infection; 5=81-100% infection. The following equation was used to calculate percentage of disease severity.

$$\text{Disease severity} = 0A + 1B + 2C + 3D + 4E + 5F / 5T \times 100$$

Where A, B, C, D, E and F are the numbers of plants corresponding to the numerical grades 0, 1, 2, 3, 4 and 5 respectively, and 5T is the total number of plants (T) multiplied by maximum disease grade 5. (Sharma *et al.*, 2006)

## 2.9. Statistical analysis:

Data were subjected to statistical analysis using analysis of variance and means were compared using least significant difference at 0.05 confidence test as described by Gomez and Gomez (1984).

## 3. RESULTS

### 3.1. Effect of KHCO<sub>3</sub> on *Sclerotium rolfsii* growth:

Growth parameters of *S. rolfsii* i.e. linear growth, number of sclerotia and MDW were variously affected by KHCO<sub>3</sub> concentrations tested (Table 1). KHCO<sub>3</sub> caused significant reduction in growth parameters and its effect was increased with increasing its concentration. The lowest KHCO<sub>3</sub> concentration (0.15 g/l) that inhibited linear growth by 33.3% sclerotial formation by 32.30% and MDW by 37.81% inhibitory effect. The highest linear growth, sclerotial formation and MDW suppression was expressed at 0.6 g/l concentration up to 76.6%, 57.69% and 95.78% inhibitory effect, respectively.

### 3.2. Effect of KHCO<sub>3</sub> on *T. harzianum* (TrA1d) growth:

Generally, growth of the bioagent *T. harzianum* was variously affected by KHCO<sub>3</sub> concentrations (Table 2). Obvious antifungal activity was pronounced by KHCO<sub>3</sub> towards the linear growth at the lowest concentration tested (0.15g/l). A significant linear growth suppression was provided due to 0.3 g/l concentration. The concentration of the inhibitory effect, since 0.6 g/l KHCO<sub>3</sub> concentration revealed a great reduction in the linear growth and MDW. Regarding spore production, KHCO<sub>3</sub> significantly inhibited spore formation and its inhibitory effect was increased with increasing KHCO<sub>3</sub> concentration.

**Table 1. Effect of potassium bicarbonate (KHCO<sub>3</sub>) on *Sclerotium rolfii* growth after incubation 7days for radial growth, mycelial dry weight and 14 days for number of sclerotia at 27°C:**

Treatments and Conc.(g/l)	Linear growth*		Sclerotia formation*		Mycelial dry weight*	
	mm/plate	Inhibition %	No. Sclerotia/plate	Inhibition %	Mg/50ml liquid media	Inhibition %
Untreated	80	0	260	0	878	0
<b>KHCO<sub>3</sub></b>						
<b>0.15</b>	53.33	33.33	176	32.30	546	37.81
<b>0.3</b>	33.33	58.33	125	51.92	94	89.29
<b>0.6</b>	18.67	76.6	110	57.69	37	95.78
<b>Means</b>	46.33	42.06	671	35.47	388	55.72
<b>L.S.D at 0.05</b>	4.50	-	9.4	-	15.60	-

\* mean of five replication

**Table 2. In Vitro effect of potassium bicarbonate (KHCO<sub>3</sub>) on linear growth and Sporulation of *Trichoderma harzianum* growth parameters after incubation for 7days, at 27°C:**

Treatment and Conc.(g/l)	Linear growth (mm)*	Sporulation (10 <sup>4</sup> /ml)*	Mycelial dry weight (mg/50ml) liquid media*
Untreated	80	153	943
<b>KHCO<sub>3</sub></b>			
<b>0.15</b>	66.71	109	876
<b>0.3</b>	55	99	701
<b>0.6</b>	40.33	74	560
<b>Means</b>	60.51	108.75	770
<b>L.S.D at 0.05</b>	3.69	3.51	39.1

\* mean of five replication

### 3.3. Effect of potassium bicarbonate (KHCO<sub>3</sub>) on *Trichoderma harzianum* antagonistic potentiality against *S.rolfsii* growth:

Table (3) showed that radial growth and sclerotial formation were significantly inhibited by KHCO<sub>3</sub> and *T.harzianum*. Highest radial growth suppression( 80% ) and sclerotial formation (76%)

was pronounced at Tri+0.6 g/l KHO<sub>3</sub> followed by (67%) and (65%) radial growth reduction, (70%) and(66%) reduction in sclerotial formation at Tri+0.3g/l KHCO<sub>3</sub> and Tri+0.15g/l KHCO<sub>3</sub>, respectively. Antagonistic potentiality of *T.harzianum* significantly enhanced when medium growth was supplemented by KHCO<sub>3</sub>

**Table 3. Effect of potassium bicarbonate (KHCO<sub>3</sub>) and *Trichoderma harzianum* on radial growth and production of sclerotia of *Sclerotium rolfii*:**

Treatments	Radial Growth*	Inhibition%	Sclerotia Formation per plate*	Inhibition%
<i>S.rolfsii</i> (control)	80	0.0	125	0.0
Tri+0.15 g/l KHCO <sub>3</sub>	28.33	65	42	66
Tri+0.3 g/l KHCO <sub>3</sub>	26	67	37	70
Tri+0.6 g/l KHCO <sub>3</sub>	16	80	29	76
<b>Means</b>	37.58	53	58.25	53
<b>L.S.D at 0.05</b>	5.14	-	4.99	-

\* mean of five replication

**3.4. Interaction effects of potassium bicarbonate (KHCO<sub>3</sub>), hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>) and *Trichoderma harzianum* on *Sclerotium rolfisii* radial growth and production of sclerotia:**

Radial growth and sclerotial formation by *Sclerotium rolfisii* were significantly inhibited by potassium bicarbonate (KHCO<sub>3</sub>), H<sub>2</sub>O<sub>2</sub> and *T.harzianum* when they tested in combinations (Table4). Antagonistic potentiality of *T.harzianum* significantly enhanced when medium growth was supplemented by (KHCO<sub>3</sub>+H<sub>2</sub>O<sub>2</sub>). However, antagonistic activity for *T.harzianum* against *S.rolfsii*

growth was increased by increasing KHCO<sub>3</sub>+H<sub>2</sub>O<sub>2</sub> concentration. Highest radial growth suppression (86.25%) and sclerotial formation (97.6%) were pronounced at Tri+1.2 g/l H<sub>2</sub>O<sub>2</sub>+0.6 KHCO<sub>3</sub> followed by (85.41%) and (73.75%) radial growth reduction, (92.8%) and (92%) reduction in sclerotial formation at Tri+1.2 g/l H<sub>2</sub>O<sub>2</sub>+0.3g/l KHCO<sub>3</sub>and Tri+1.2 g/l H<sub>2</sub>O<sub>2</sub>+0.15 KHCO<sub>3</sub>, respectively. Since at Tri+0.8 H<sub>2</sub>O<sub>2</sub> g/l +0.6g/l KHCO<sub>3</sub> exhibited inhibitory effects being (84.16%) radial growth, (90.4%)in sclerotial formation while at Tri+1.2g/l H<sub>2</sub>O<sub>2</sub>+0.6KHCO<sub>3</sub> gave more inhibitory effects

**Table 4. Effect of potassium bicarbonate (KHCO<sub>3</sub>), hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>) and *Trichoderma harzianum* on *Sclerotium rolfisii* radial growth and production of sclerotia:**

Treatments	Radial growth	Inhibition%	Sclerotia	
			formationper plate	Inhibition%
<i>S.rolfsii</i> (control)	80	0.0	125	0.0
Tri+0.8 g/l H <sub>2</sub> O <sub>2</sub> +0.15 g/l KHCO <sub>3</sub>	30	62.5	28	77.6
Tri+0.8 g/l H <sub>2</sub> O <sub>2</sub> +0.3 g/l KHCO <sub>3</sub>	28	65	23	81.6
Tri+0.8 g/l H <sub>2</sub> O <sub>2</sub> +0.6 g/l KHCO <sub>3</sub>	12.67	84.16	12	90.4
Tri+1.2g/l H <sub>2</sub> O <sub>2</sub> +0.15 g/l KHCO <sub>3</sub>	21	73.75	10	92
Tri+1.2g/l H <sub>2</sub> O <sub>2</sub> +0.3 g/l KHCO <sub>3</sub>	11.67	85.41	9	92.8
Tri+1.2g/l H <sub>2</sub> O <sub>2</sub> +0.6 g/l KHCO <sub>3</sub>	11	86.25	3	97.6
Means	27.76	65.29	30	76
L.S.D at 0.05	5.14	-	4.99	-

**3.5. Management of sunflower root/collar rot:**

In pot experiments, pre sowing soaking seeds in KHCO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> and *Trichoderma harzianum* individually or in combinations solution resulted in increasing resistance of sunflower plants against *S.rolfsii* infection (Table 5). A significant reduction in diseases severity was obtained by 0.15 g/l KHCO<sub>3</sub> concentration (66.66%) was increased with

increasing KHCO<sub>3</sub> concentration (46%) at 0.6 g/L KHCO<sub>3</sub> , As for interaction effects, all combinations tested provided significant protection enhancement as compared to the untreated control. The highest protection (75%) was provided when seeds were treated by (Tri+1.2g/l H<sub>2</sub>O<sub>2</sub>+0.6g/l KHCO<sub>3</sub>) followed by (71%) when seeds were treated by (Tri+1.2g/l H<sub>2</sub>O<sub>2</sub>+0.3 g/l KHCO<sub>3</sub>).

**Table 5. Interaction effects of H<sub>2</sub>O<sub>2</sub>, KHCO<sub>3</sub> and *T. harzianum* on the severity of sunflower root rot caused by *S.rolfsii* under field conditions during 2019 and 2020 growing seasons:**

Treatment and Conc.(g/l)	Disease severity%		Mean	Protection%
	2019	2020		
Untreated	100	100	100	0
KHCO <sub>3</sub> 0.15 g/l	70	63.33	66.66	33.34
KHCO <sub>3</sub> 0.3 g/l	58	53.66	55.83	44.17
KHCO <sub>3</sub> 0.6 g/l	52	40	46	54
H <sub>2</sub> O <sub>2</sub> 1.2 g/l	68	33.33	50.66	49.34
<i>Trichoderma harzianum</i> (Tri)	48	53.66	50.83	49.17
0.6 g/l KHCO <sub>3</sub> +1.2 g/l H <sub>2</sub> O <sub>2</sub>	38	40	39	61
Tri+1.2g/l H <sub>2</sub> O <sub>2</sub> +0.3 g/l KHCO <sub>3</sub>	33	25	29	71
Tri+1.2g/l H <sub>2</sub> O <sub>2</sub> +0.6 g/l KHCO <sub>3</sub>	30	20	25	75
L.S.D at 0.05	Treatments (A): 12.68	Growing seasons (B): 5.20	(A×B): 18.49	

#### 4. DISCUSSION

*Sclerotium rolfsii*, the causal agent of collar/root rot of many crops *Sclerotium rolfsii* is soil and borne plant pathogenic fungus that attacking more than 500 of plant species belonging to over 100 families (Bilah *et al.*, 2017). Synthetic fungicides are used to control soil borne pathogens on vegetable growing areas all over the world as well as the development of fungicide resistant strains of pathogens have created the need to find alternatives to pesticides (Arslan *et al.*, 2009 and Erper *et al.*, 2011). The fungicidal efficacy of salts direct and indirect effects of PH on sclerotial germinations were tested in vitro experiments against a soilborne pathogen, *Sclerotium rolfsii* Sacc.(Punja and Grogan,1982). Many investigations reported the use of potassium salts ( $K_2HPO_4$ ,  $KNO_3$ ) as a chemical agent for induction of plant resistance (Stromberge and Brishammar, 1991and Yurina *et al.*,1993).

$KHCO_3$  can greatly promote photosynthetic rate and stomatal conduction of leaves, significantly increase leaf area, contents of soluble sugar, protein, chlorophyll and improve photosynthesis rate of plants.  $KHCO_3$  could improve stomatal conductance and electron transport activity (Lei *et al.*, 2009 and Li and Hao, 2013).  $H_2O_2$  acts as a messenger molecule involved in adaptive signaling, triggering tolerance against various abiotic stresses at low concentrations, but at high concentrations it orchestrates programmed cell death. Usually, abiotic stress, such as drought, will increase the production of ROS in the plant.  $H_2O_2$  at lower level can have a significant positive effect on plant growth, growth regulators, antioxidant enzyme activity, fruit yield and quality of tomato.the exogenous application of  $H_2O_2$  increased the plant growth, physiological activities and biochemical properties of wax apple fruits (Khandaker *et al.*,2012 and Orabi *et al.*,2015)

Furthermore, there has been considerable interest in the use of sodium bicarbonate, potassium bicarbonate and potassium phosphate for controlling various fungal disease in plants (Karabulut *et al.*, 2003 and Smilanick *et al.*, 2005;E-Mougy *et al.*,2013). As result, the application of bicarbonates represents an effective technique to control fungal pathogens for horticultural crops (Aharoni *et al.*, 1997;Bombeli and Wright 2006; Aguitar *et al.*,2011 and Khiareddine *et al.*,2016).

Potassium bicarbonate, hydrogen peroxide and biocontrol agent *T. harzianum* were concerned individually or in combination to minimize *S.rolfsii*

growth and infectivity. *In vitro* studies showed that  $KHCO_3$ was effective to suppress linear growth, mycelial dry weight and sclerotia production which recorded the highest inhibition , 76.6, 57.69 and 95.78%, respectively at 0.6g/l  $KHCO_3$ . The bicarbonate causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan,1982 and Ziv and Zitter,1992).On the other hand the role of potassium bicarbonate in increasing crop resistance to disease caused by bacteria and fungi was widely reviewed by Perrenoud(1990).

The highest inhibition percentages were recorded for radial growth sclerotial formation at Tri+0.6 g/l  $KHCO_3$  80% and 76%, respectively. Moreover, antagonistic potentiality for *T. harzianum* was affected by  $KHCO_3$ . Potassium bicarbonate provided synergistic effects with *T. harzianum* since the antagonistic potentiality of *T. harzianum* against *S.rolfsii* was increased with increasing  $KHCO_3$  concentration.

The growth inhibition of root rot fungi by dual culture in this study could be due to its fast growing nature, secretions of armful extra-cellular compounds like antibiotics, cell wall degrading enzymes such as  $\beta$ - 1,3 gluconase, endochitinase and chitinase enzymes which degrade the cell wall leading to lyase of mycelium of the pathogen and mycoparasitism in dual culture as found with other fungi (Rahman *et al.*,2010; Sallam Nashwa *et al.*, 2008)

The sodium bicarbonate and hydrogen peroxide combination prevented bacterial growth of *S. mutans*. The results show that products containing these agents have the ability to stop the growth of *S. mutans*.(Silhacek and Taake, 2005)

This work showed that potassium bicarbonate combined with hydrogen peroxide and *T. harzianum* to maximize their effect on sunflower root/collar rot control . *In vitro* studies showed that using Tri+1.2 g/l  $H_2O_2$ +0.6g/l  $KHCO_3$  recorded the highest inhibition in radial growth and sclerotial formation 86.25, 92.8%, respectively followed by 85.41, 97.6% respectively at concentration Tri+1.2 g/l  $H_2O_2$ +0.3g/l  $KHCO_3$  . In pot experiments,  $KHCO_3$ ,  $H_2O_2$  and *T. harzianum* showed significant reduction in sunflower basal stem rot caused by *S.rolfsii*. Even at using these components individually. Using 1.2 g/l  $H_2O_2$  appeared protection (49.34%) value followed by 0.6 g/l  $KHCO_3$  54%, However. The highest protection recorded in the combinations *T. harzianum*+1.2 g/l  $H_2O_2$  +0.6g/l  $KHCO_3$  (75%)(EL-Mougy *et al.*, 2013 and Erper *et al.*, 2011)

## 5. CONCLUSIONS

Basal stem rot caused by *Sclerotium rolfsii* Sacc., is one of the most important diseases affecting Sunflower crop all over world. Presently, greater emphasis has been replaced with biological control, in order to reduce the environmental hazards, to avoid the development of resistant strains and to reduce the cost of cultivation. Combination with bio control agents and potassium bicarbonate (KHCO<sub>3</sub>) or hydrogen peroxide was effective against suppressing the pathogen rather than following only the potassium bicarbonate (KHCO<sub>3</sub>)

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## الملخص العربي

### مكافحة عفن ساق وجذور عباد الشمس المتسبب عن فطر *Sclerotium rolfsii* باستخدام الطرق الكيميائية والحيوية

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أظهرت الدراسات المعملية تأثير معنوي مثبت لبكتريونات البوتاسيوم علي قياسات النمو كل من الفطر الممرض *Sclerotium rolfsii* المسبب لعفن ساق وجذور عباد الشمس . التأثيرات التثبيطية ضد الفطر لها تأثير طردي مع زياده تركيزات بيكربونات البوتاسيوم. بيكربونات البوتاسيوم لها تأثير معنوي ضد *Sclerotium rolfsii* حيث كانت النتائج ٧٦.٦، ٥٧.٦٩% و ٩٥.٧٨% تثبيط للنمو الخطي وتكوين الاجسام الحجرية والوزن الجاف للفطر علي التوالي باستخدام تركيز ٠.٦ جرام / لتر بيكربونات بوتاسيوم. بيكربونات البوتاسيوم أعطت تأثير معنوي مع الكائن الحيوي *Trihoderma harzianum* وضد فطر الكائن الحيوي مع *Sclerotium rolfsii* زاد بزيادة تركيز بيكربونات البوتاسيوم (٠.٦ جرام / لتر) حيث أعطت النتائج نسبة تثبيط ٨٠% و ٧٦% للنمو الخطي وتكوين الاجسام الحجرية علي التوالي ومع ذلك ، فإن الأكثر فعالية لمنع قياسات نمو وفعالية فطر *Sclerotium rolfsii* هي توليفات من بيكربونات البوتاسيوم و فوق أكسيد الهيدروجين مع الكائن الحيوي *Trihoderma harzianum* حيث أعطت النتائج نسبة تثبيط ٨٦.٢٥% و ٩٧.٦٠% للنمو الشعاعي وتكوين الأجسام الحجرية عند تركيز ١.٢ جرام / لتر من فوق أكسيد الهيدروجين مع ٠.٦ جرام / لتر بيكربونات بوتاسيوم مع الكائن الحيوي *Trihoderma harzianum* على التوالي. أظهرت النتائج أن تركيز فوق أكسيد الهيدروجين ١.٢ جرام / لتر مع بيكربونات البوتاسيوم ٠.٦ جرام / لتر مع الكائن الحيوي *Trihoderma harzianum* أعطي حماية من الاصابة بعفن ساق وجذور عن فطر *Sclerotium rolfsii* بنسبة ٧٥% يليها تركيز فوق أكسيد الهيدروجين ١.٢ جرام / لتر مع بيكربونات البوتاسيوم ٠.٣ جرام / لتر مع الكائن الحيوي *Trihoderma harzianum* أعطي حماية من الاصابة بعفن ساق وجذور عن فطر *Sclerotium rolfsii* بنسبة ٧١%.