African Journal of Biotechnology Vol. 10(43), pp. 8605-8612, 10 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.411
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Evaluation of *in vitro* antifungal activity of potassium bicarbonate on *Rhizoctonia solani* AG 4 HG-I, *Sclerotinia sclerotiorum* and *Trichoderma* sp.

Ismail Erper[1]*, Muharrem Turkkan[2], Gürsel H. Karaca[3] and Gülay Kılıc[1]

[1]Plant Protection Department, Ondokuz Mayis University, Samsun, Turkey.
[2]Plant Protection Department, Ordu University, Ordu, Turkey.
[3]Plant Protection Department, Süleyman Demirel University, Isparta, Turkey.

Accepted 15 June, 2011

The effect of increased concentrations of potassium bicarbonate ($KHCO_3$) as a possible alternative to synthetic fungicides for controlling *Rhizoctonia solani* AG 4 HG-I and *Sclerotinia sclerotiorum* was evaluated *in vitro,* in this study. In addition, the effect of potassium bicarbonate on *Trichoderma* sp., a natural antagonist on *R. solani* AG 4 HG-I and *S. sclerotiorum* was determined. Potassium bicarbonate substantially inhibited ($P<0.05$) the growth of the three fungal strains. Mycelial growth of *R. solani* AG 4 HG-I significantly decreased as the concentration of bicarbonate increased, especially at concentrations greater than 200 mM. Similarly, mycelial growth of both *S. sclerotiorum* and *Trichoderma* sp. dramatically reduced in increasing concentrations of $KHCO_3$. Mycelial growth of either fungi was completely inhibited when exposed to 100 mM bicarbonate. In addition, $KHCO_3$ concentrations higher than 10 mM caused significant (*P<0.05*) reduction of the sclerotium formation of *S. sclerotiorum*. Also, sclerotium germination and *de novo* sclerotium formation were significantly inhibited as the concentrations of $KHCO_3$ increased. As a result, it was concluded that potassium bicarbonate was an alternative chemical agent for controlling *R. solani* AG 4 HG-I and *S. sclerotiorum.* Also, $KHCO_3$ was found to have negative effects on *Trichoderma* sp.

Key words: Antifungal effect, $KHCO_3$, soil borne pathogens, sclerotium germination.

INTRODUCTION

The soilborne fungi, agents of root rot and damping off diseases, cause important economic losses on various crops that often results in the death of plants every year (Van Bruggen et al., 1986; Villajuan-Abgona et al., 1996). *Rhizoctonia solani* Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary are major pathogens on different plants. *R. solani* AG 4 is the most common pathogen among anastomosis groups (AG) worldwide, causing damping-off, stem rot and root rot on bean, soybean, faba bean, cowpea and pea (Galindo et al., 1983; Sneh et al., 1991). AG 4 has been divided into subgroups: AG 4 HG-I, AG 4 HG-II and AG 4 HG-III (Kuramae et al., 2003). Especially, among *R. solani* anastomosis groups, AG 4 and AG 4HG-I subgroup are the most common fungal pathogens in bean growing areas in the Black Sea Region of Turkey (Karaca et al., 2002; Cebi, 2009; Erper et al., 2011).

S. sclerotiorum is an agressive and destructive fungal pathogen that causes rot root and wilting on different host plants (Bolton et al., 2006), and is known to attact over 480 species of host plants worldwide in many different soil types and environmental conditions (Boland, 1990; Dillard and Cobb, 1995). The fungus survives in soil as sclerotia, which are hardened structures of fungal mycelium (Ben-Yephet et al., 1993) and a viable source of inoculum for long periods under unfavorable conditions (Wu and Subbarao, 2006; Bae and Knudsen, 2007).

In controlling the damage of soilborne pathogens,

*Corresponding author. E-mail: ismailer@omu.edu.tr. Tel:+90 362 3121919. Fax: +90 362 4576034.

several agronomic practices have been implemented such as crop rotation, resistant varieties, soil fumigation, biological control and pesticide applications (Bae and Knudsen, 2007). Among these practices, the management of soilborne pathogens with chemicals is most difficult because of their extremely wide host spectrum and the high survival rate of resistant forms such as sclerotia under different environmental conditions (Yangui et al., 2008). In addition, chemicals have negative side effects on the environment and their application to soil is difficult and expensive (Ozkoc et al., 2002). Synthetic fungicides are used to control soilborne pathogens on vegetable growing areas all over the world. In recent years, public demands to reduce pesticide use, stimulated by greater awareness of environmental and health issues as well as the development of fungicide resistant strains of pathogens, have created the need to find alternatives to pesticides (Arslan et al., 2009).

One way to achieve this aim may be the use of efficient natural substances such as salt compounds. The main advantages of using salt compounds include their relatively low mammalian toxicity, a broad spectrum of modes of action and relatively low cost (Olivier et al., 1998). Bicarbonates are widely utilized to regulate pH, to avoid undesirable fermentation processes, and to improve texture and taste in the food industry (Aharoni et al., 1997; Smilanick et al., 1999). They, also have wide-spectrum antimicrobial properties. Therefore, they have been successfully tested especially in the control of postharvest fungal pathogens by different studies. Some bicarbonates (potassium, sodium or ammonium) and carbonates have been demonstrated to prevent the growth of several fungal pathogens after harvest (Palmer et al., 1997; Olivier et al., 1998; Palou et al., 2001; Karabulut et al., 2003; Jamar et al., 2007). There are some investigations that are related to the use of carbonates against soil pathogens in recent years. In a study which was carried out in order to evaluate the efficacy of eight food additives for the control of four soilborne pathogens, ammonium bicarbonate was used to control all fungi at 2%, which is the highest concentration used in this study (Arslan et al., 2009). In another study, the potential benefits of potassium bicarbonate for controlling both growth and development of *S. sclerotiorum* were evaluated, although, it also exerts negative effects on the *Trichoderma* strain that is a natural antagonist to *S. sclerotiorum* (Ordonez et al., 2009). Similar assessments were made *in vitro* against *Botrytis cinerea* colony growth in response to ammonium, potassium and sodium bicarbonates, and bicarbonates inhibited colony growth at concentrations as low as 20 mM (Palmer et al., 1997). In the same manner, potassium carbonate and sodium bicarbonate totally inhibited the growth of crater rot of *Daucus carota* caused by *Rhizoctonia carotae* which were fungicidal (Ricker and Punja, 1991). In addition, the fungicidal efficacy of salts and direct and indirect effects of pH on sclerotial germinations were tested in *in vitro* experiments against a soilborne pathogen, *Sclerotium rolfsii* Sacc. (Punja and Grogan, 1982). As a result, the application of bicarbonates represents an effective technique to control fungal pathogens for horticultural crops (Aharoni et al., 1997; Bombelli and Wright 2006).

However, biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Eziashi et al., 2007). *Trichoderma* is a natural fungal genus that may be saprophytic or mycoparasitic, producing antifungal metabolites which may compete, inhibit, or cause lysis of several structures of plant fungal pathogens (Benítez et al., 2004). For instance, *Trichoderma* species are antagonists that inhibit and parasitize either mycelium or sclerotia of different *Sclerotinia* species (Bolton et al., 2006; Abdullah et al., 2008) and *R. solani* (Chet and Baker, 1981; Elad et al., 1983).

Efficient results were obtained in previous studies carried out on biological control of *R. solani* and *S. sclerotiorum* with *Trichoderma* species. The efficacy of bicarbonates to control postharvest and soilborne pathogens has been successfully tested. But, there is not enough information especially about the response of both *R. solani* AG 4 HG-I and *S. sclerotiorum*, or their interactions with *Trichoderma* sp., when exposed to potassium bicarbonate.

The objective of this study was to evaluate the effect of increased concentrations of potassium bicarbonate as possible alternatives to synthetic fungicides for controlling *R. solani* AG 4 HG-I and *S. sclerotiorum in vitro.* Similarly, the effect of potassium bicarbonate on *Trichoderma* sp. strain was determined.

Firstly, the individual growth of the three fungi and the interaction of both *R. solani* AG 4 HG-I- *Trichoderma* sp. and *S. sclerotiorum-Trichoderma* sp. were examined, secondly *de novo* sclerotium formation by *S. sclerotiorum* was examined.

MATERIALS AND METHODS

Fungal cultures

All fungal cultures used in this study including *R. solani* AG 4 HG-I, *S. sclerotiorum* and *Trichoderma* sp. were originally isolated from bean growing areas in Black Sea Region of Turkey during routine disease surveys in 2006 and 2008. *S. sclerotiorum* Ss-5 isolate were isolated from diseased bean plants showing signs of white rot and identified according to its cultural features. For this study, Thz-23 isolate was selected among 24 effective strains belonging to *Trichoderma* genus, with *in vitro* mycoparasitic activity on virulent *R. solani* AG 4 HG-I and *S. sclerotiorum* and it was identified by Dr. Irina S. Druzhinina, Institute of Chemical Engineering, Vienna University of Technology, Austria. *R. solani* AG 4 HG-I, M-62 isolate used in the experiment, which causes root rot disease on bean plants was provided by Dr. M. Cebi (Ondokuz Mayis University, Faculty of Arts and Science, Biology Department, Samsun, Turkey). Cultures of each of the fungi were maintained on potato dextrose agar (PDA: Oxoid). The PDA slants were stored at +4°C and served as stock cultures for further use.

Assesment of mycelial growth

Different potassium bicarbonate concentrations on the following basis: 0, 2, 4, 6, 8, 10, 25, 50, 75, or 100 mM (KHCO3, Carlo Erba Reagenti (Milan-Italy) were added to autoclaved and cooled PDA medium at 500C for *S. sclerotiorum* and *Trichoderma* sp. The pH of each KHCO3 concentration was 6.0, 7.0, 7.0, 7.1, 7.2, 7.3, 8.0, 8.1, 8.1, and 8.3, respectively For *R. solani* AG 4 HG-I, potassium bicarbonate concentrations on the following basis 0, 10, 25, 50, 75, 100, 150, 200, 500, or 750 mM. were used in the experiment and the pH of each KHCO3 concentration was 6.0, 7.3, 8.0, 8.1, 8.1, 8.3, 8.3, 8.3, 8.4, and 8.4, respectively. The medium was dispensed aseptically into 9-cm-diameter Petri plates. For three the fungi, a mycelial disc (5 mm diameter) taken from 7-day-old culture that was grown on PDA was placed in the center of each potassium bicarbonate-amended PDA. The plates were then sealed with parafilm and incubated at 25°C, and an approximate photoperiod of 12 h for 6 days. The growth of each fungal colony was measured daily. At the end of the experiment, the number of sclerotia on *S. sclerotiorum* cultures was also determined (Ordonez et al., 2009). All experiments were conducted twice.

Experimental design and data analysis

All experiments were conducted in a complete randomized designs with ten treatments and three replications. Analysis of variance was implemented using the program MINITAB and Duncan at 0.05 significance level was used to compare treatment means.

Confronted tests *in vitro*

In this study, *R. solani* AG 4 HG-I-*Trichoderma* sp. and *S. sclerotiorum*-*Trichoderma* sp. were evaluated *in vitro* by placing both fungi on opposite sides of 9-cm diameter Petri dishes including PDA amended with several concentrations of $KHCO_3$ as previously described in triplicate. Fungal cultures were incubated as already described, and the antagonism of *Trichoderma* sp. against AG 4 HG-I isolate and *S. sclerotiorum* isolate was visually examined after 4 days (Ordonez et al., 2009). Also, the interaction between each fungi was examined by using an Olympus CX-31 compound microscope at 100 to 400x magnification. All experiments were conducted twice.

Assesment of sclerotia germination and *de novo* formation

Sclerotia were obtained from pure cultures of *S. sclerotiorum* that were grown on PDA. Sclerotia were collected, placed in sterile microtubes, and kept at 5°C until they were used. Then, sclerotia were surface disinfested to avoid proliferation of bacterial contaminants by placing them in 70% ethanol for 40 s, and rinsed in sterile-distilled water (SDW), adding a solution of streptomycine (200 µg/ml), and keeping them at 4°C overnight and blotted dry on sterile paper towels to eliminate excess antibiotic solution. Then, once dried, ten sclerotia were placed on 9-cm diameter Petri dishes containing PDA with the concentrations of $KHCO_3$ as mentioned earlier.

The Petri dishes were incubated at 25°C for 11 days, and the number of germinated sclerotia was daily recorded for each treatment, in addition to the number of newly formed sclerotia (Ordonez et al., 2009). All experiments were conducted twice.

Similarly, the experiments were conducted in a complete randomized designs with ten treatments and three replications. Analysis of variance was implemented using the program MINITAB and Duncan at 0.05 significance level was used to compare treatment means.

RESULTS

Mycelial growth

Potassium bicarbonate significantly inhibited the mycelial growth of each three fungi ($P<0.05$). *R. solani* AG 4 HG-I (isolate M-62) started growing at 24 h, but its growth significantly decreased ($P<0.05$) with the increasing bicarbonate concentrations (Figure 1), especially at concentrations greater than 200 mM. At 72 h, it was observed that complete surface of the PDA was covered by *R. solani* AG 4 HG-I at concentrations from 0 to 25 mM. After 48 h, at 50 mM $KHCO_3$, the mycelium growth of M-62 isolate was reduced 50% as compared to the control (0 mM). Similarly, after 72 h, the mycelium growth of M-62 isolate cultured on 75 mM potassium bicarbonate amended media decreased 60% compared to 0 mM. On the other hand , no growth was observed at 750 mM concentration of $KHCO_3$.

Similarly, it was observed that *S. sclerotiorum* started growing after 24 h. However the mycelial growth of *S. sclerotiorum* significantly reduced with the increasing concentrations of $KHCO_3$. Its mycelia covered the petri dishes nearly at 0 and 4 mM concentrations of bicarbonate after 48 h, whereas it completely covered the petri dishes after 72 h at 0 to 8 mM bicarbonate concentrations. Mycelial growth of the fungus was inhibitited especially at concentrations greater than 25 mM. Moreover, it completely inhibited when exposed to 100 mM bicarbonate (Figure 2).

In this study, it was observed that sclerotium formation by *S. sclerotiorum* was also inhibited at increasing $KHCO_3$ concentrations. At the end of the fifth day, sclerotia formation was observed at concentrations from 0 to 8 mM, but sclerotia were not formed at other bicarbonate concentrations. Only the presence of white-cotton structures of aggregate hyphae was observed. At the end of 144 h, the number of sclerotia formed ranged between 5.3 and 16.7 at concentrations from 0 to 10 mM (Figure 3).

As compared with both fungal pathogens, *Trichoderma* sp. started growing after 24 h and its mycelial growth was dramatically reduced ($P<0.05$) with the increasing bicarbonate concentrations. In addition, after 48 h mycelial growth of *Trichoderma* sp. cultured on 8 mM $KHCO_3$ amended media decreased 50% as compared to control treatment (0 mM) . No fungal growth was observed at 100 mM bicarbonate (Figure 4).

Confronted tests

According to the results of the fungal confrontation test, *Trichoderma* sp. Thz-23 isolate was determined to have antagonistic effect on both *R. solani* AG 4 HG-I M-62 isolate and *S. sclerotiurum* Ss-5 isolate when exposed to increasing concentrations of $KHCO_3$.

It was determined with microscobic observations that

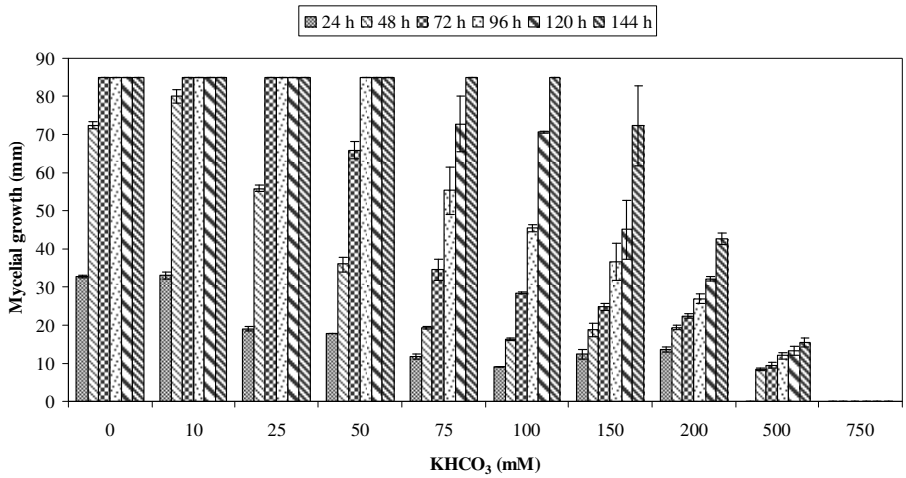

Figure 1. Time relevant *in vitro* mycelial growth of *Rhizoctonia solani* AG 4 HG-I exposed to potassium bicarbonate ($KHCO_3$). Vertical lines represent standard errors of the means.

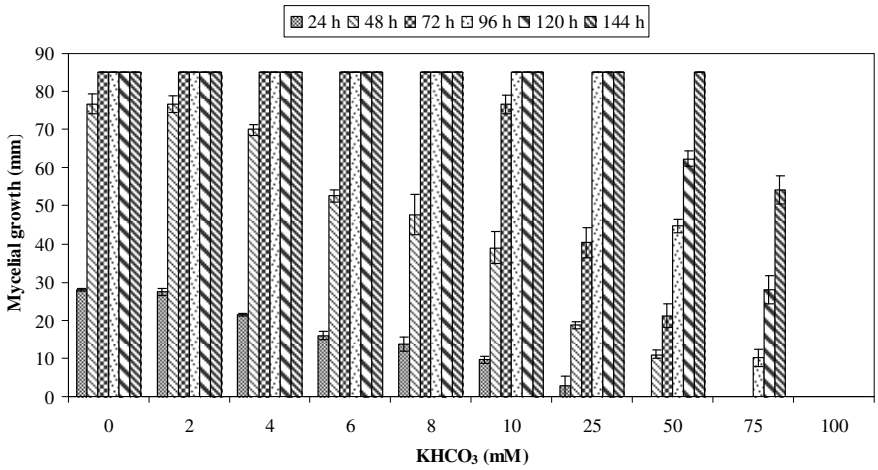

Figure 2. Time relevant *in vitro* mycelial growth of *Sclerotinia sclerotiorum* exposed to potassium bicarbonate ($KHCO_3$). Vertical lines represent standard errors of the means.

Thz-23 isolate growed on hyphae of M-62 isolate and showed mycoparasitic effect when exposed to 0-25 mM concentrations of $KHCO_3$. It was observed at 100 to 400´ microscope magnification that hyphae of Thz-23 isolate form dense coils and tightly encircled the hyphae of M-62 isolate. Hyphae of the M-62 isolate lost its turgor and cells collapsed, whereas Thz-23 isolate hyphae seemed to be normal.

In contrast, the interaction between Thz-23 isolate and M-62 isolate was not observed especially due to inhibition of Thz-23 isolate when exposed to 100 mM bicarbonate. As a result of the confrontation test of Thz-23-Ss-5, almost similar results were obtained. Thz-23 isolate showed antagonistic effect against Ss-5 isolate with the increasing bicarbonate concentrations. However, growth of both fungi inhibited when exposed to 75 mM bicarbonate. As a result, fungal confrontation between these fungi was not observed at concentrations of bicarbonate higher than 75 mM.

Sclerotia germination and *de novo* formation

Similarly, it was observed that potassium bicarbonate applications affected sclerotia germination. Firstly, at 48 h sclerotia germination were determined from 0 to 6 mM $KHCO_3$ concentrations, but 100% germination was occurred at conrtol (0 mM) after 4 days (Figure 5).

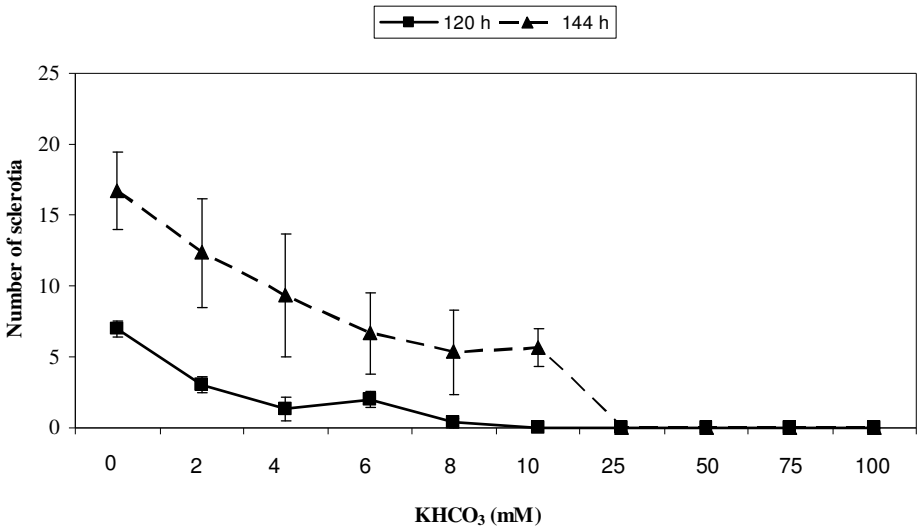

Figure 3. Effect of potassium bicarbonate ($KHCO_3$) concentrations on the number of sclerotia of *Sclerotinia sclerotiorum*, after 120 and 144 h. Vertical lines represent standard errors of the means.

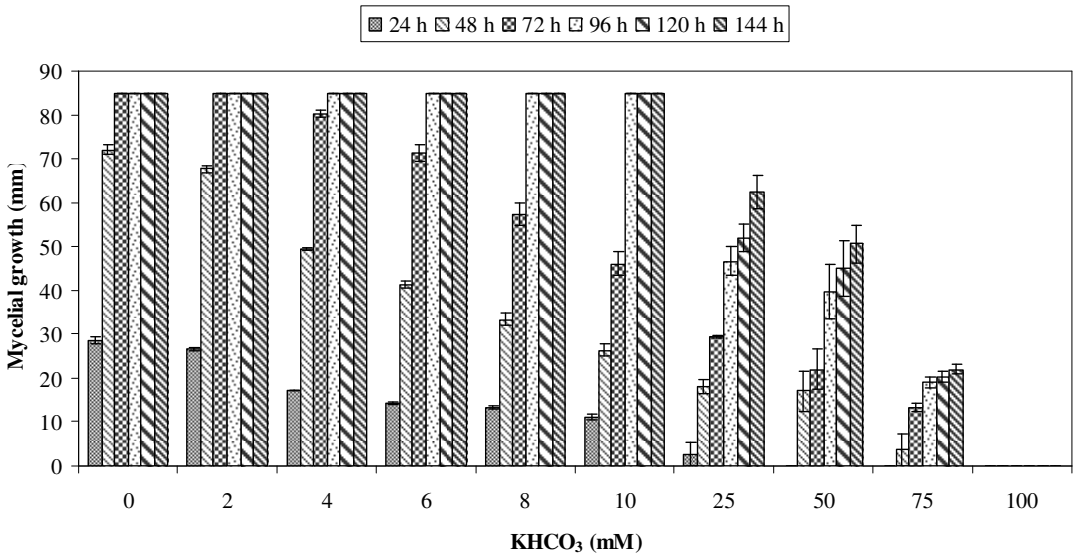

Figure 4. Time relevant *in vitro* mycelial growth of *Trichoderma* sp. exposed to different concentrations of potassium bicarbonate ($KHCO_3$). Vertical lines represent standard errors of the means.

However, at the end of seven days all sclerotia germinated from at concentration from 0 to 25 mM, but sclerotia germination did not occur in 100 mM $KHCO_3$ concentration. In addition, the production of de novo sclerotia by bicarbonate application significantly decreased ($P<0.05$). After the sixth day, de novo sclerotia were formed in the from 0 to 6 mM concentrations. At the 11th day, while the de novo sclerotia formation were observed from 0 to 25 mM, at between 50 to 100 mM bicarbonate application did not formed sclerotia (Figure 6).

DISCUSSION

To our knowledge, this is the first report on the antifungal activity of potassium bicarbonate against isolate of *R. solani* AG 4 HG-I which is one of the major fungal pathogens of bean plants in the Black Sea Region, Turkey. This study demonstrated that different concentrations of potassium bicarbonate significantly inhibited *in vitro* mycelial growth of *R. solani* AG 4 HG-I, *S. sclerotiorum* and *Trichoderma* sp.. Among three fungi isolated from infected bean plants, *S. sclerotiorum* and

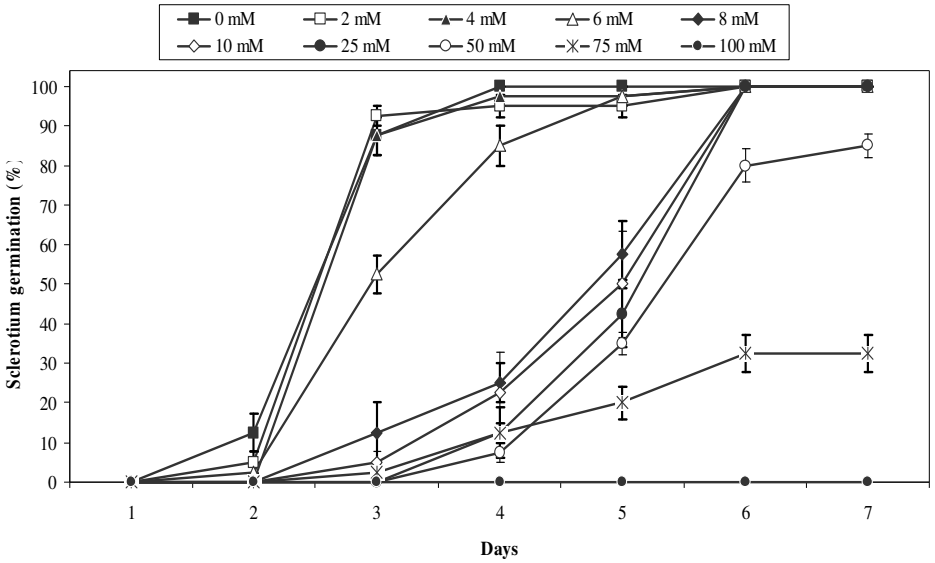

Figure 5. Germination (%) of the sclerotia of *Sclerotinia sclerotiorum* exposed to different concentrations of potassium bicarbonate (KHCO3) after 168 hourtime *in vitro*. Vertical lines represent standard errors of the means.

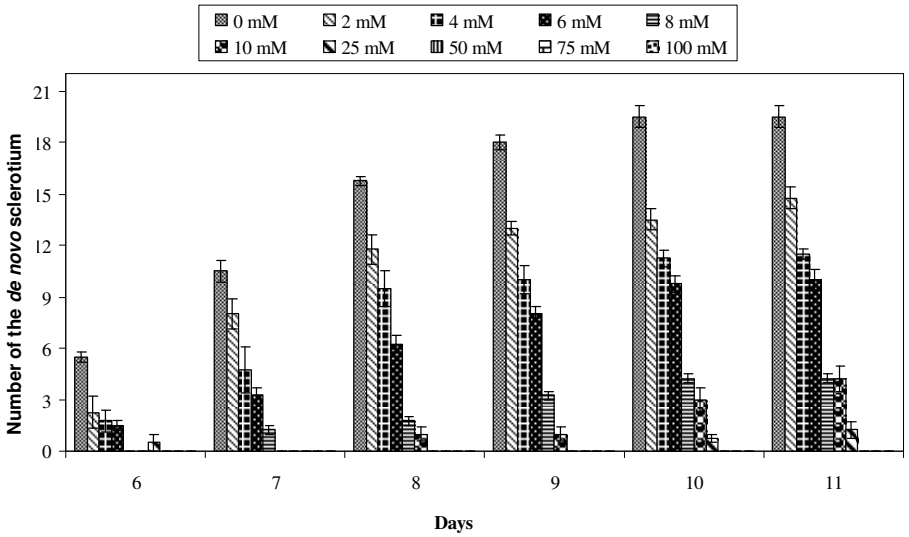

Figure 6. Effects of increasing concentrations of potassium bicarbonate ($KHCO_3$) on de novo sclerotium formation by *Sclerotinia sclerotiorum* after the fifth days of sclerotium germination. Vertical lines represent standard errors of the means.

Trichoderma sp. were affected from the lower concentrations of $KHCO_3$ more than *R. solani* AG 4 HG-I isolate. Mycelial growth of *S. sclerotiorum* and *Trichoderma* sp. showed nearly 35% and 74% reduction after 144 h at 75 mM concentration of bicarbonate. Both fungi showed no sign of growth at 100 mM concentration of the chemical, while *R. solani* AG 4 HG-I isolate grew at 500 mM bicarbonate concentration. Thus, these growth responses indicated that both *S. sclerotiorum* and *Trichoderma* sp. were more sensitive to increasing $KHCO_3$ concentrations than *R. solani* AG 4 HG-I isolate. However, mycelial growth of this isolate was also affected by the increasing concentrations of bicarbonate and it significantly decreased especially at concentrations higher than 200 mM. It was found in the study that mycelial growth of M-62 isolate cultured on 50 mM $KHCO_3$ amended media decreased 50% after 48 h, compared to control treatment (0 mM). Similarly, Arslan et al.,(2009) reported 50% reduction (ED_{50}) on mycelial growth of *R. solani* AG-4 at 0,35% (w/v) concentration of

$KHCO_3$. Ricker and Punja (1991) evaluated the effects of nine fungicides and two chemical salts (potassium carbonate-K_2CO_3 and sodium bicarbonate-$NaHCO_3$) on mycelial growth of crater rot agent, *Rhizoctonia carotae*, and determined that two chemical salts totally inhibited the growth of *R. carotae* at 0,1 M and had fungicidal effects.

The results of the present study indicated that both *S. sclerotiorum* and *Trichoderma* sp. did not grow at $KHCO_3$ concentrations greater than 75 mM. Similarly, Ordonez et al.,(2009) detected the antifungal effect of increasing potassium bicarbonate concentrations on *Trichoderma* sp. strain R39 and *S. sclerotiorum.* In the same study, $KHCO_3$ exerted a substantial inhibitive effect on the mycelial growth of both fungi, although either fungus behaved differentially when exposed to the various concentrations of bicarbonate. *Trichoderma* sp. showed a 38.8% reduction of growth at 50 mM after 144 h, when compared to its respective control (0 mM). *S. sclerotiorum* had an average growth reduction of 63.9% at low and intermediate bicarbonate concentrations, but this pathogen did not show any mycelial growth at 50 mM. This study indicated that *Trichoderma* sp. (Thz-23 isolate) was more sensitive to high concentrations of bicarbonate than *S. sclerotiorum.* It was observed that *Trichoderma* sp. growth was inhibited 40% at 50 mM when compared to control whereas *S. sclerotiorum* totally covered the surface of the petri dishes at 144 h at 50 mM. Different results between these similar studies may be due to variation of virulence or discrepancies of both *S. sclerotiorum* isolates. This finding, also, confirmed our results that increasing $KHCO_3$ concenatractions prevented the mycelial growth of *Trichoderma* sp.

Previous studies reported that potassium bicarbonate and other bicarbonate salts significantly inhibited *in vitro* growth of various plant pathogens (Punja and Grogan 1982; Punja and Gaye 1993; Palmer et al., 1997; Mills et al., 2004; Oliver et al., 1998; Arslan et al., 2009; Ordonez et al., 2009). Punja and Grogan (1982) reported that bicarbonate salts ($KHCO_3$) prevented sclerotial germination of *Sclerotium rolfsii*, but only carbonate and bicarbonate salts were showed fungicidal effect. In another study, Punja and Gaye (1993) reported that ammonium bicarbonate, calcium propionate, potassium carbonate, potassium sorbate or sodium bicarbonate reduced black root rot caused by *Chalara elegans* on carrots. Oliver et al., (1998) reported that totally seven potassium bicarbonate salt compounds reduced the severity of silver scurf caused by *Helminthosporium solani* on potato tubers. Similarly, Arslan et al., (2009), investigated the efficacy of eight food additives as possible alternatives to synthetic fungicides for the control of soilborne pathogens; *Fusarium oxysporum* f. sp. *melonis*, *Macrophomina phaseolina*, *R. solani*, and *S. sclerotiorum*, and the fungistatic effect of $KHCO_3$ was detected at 0.2-0.8% (w/v) concentration. Another study evaluated *in vitro* antifungal effect of increasing $KHCO_3$ concentrations on *S. sclerotiorum* and *Trichoderma* sp. strain R39, and results showed potential benefits of potassium bicarbonate for controlling *S. sclerotiorum*, although it also exerted negative effects on *Trichoderma* sp. that is a natural antagonist of *S. sclerotiorum* (Ordonez et al., 2009).

Soil fungi are more active under acidic pH values (Ordonez et al., 2009). Previous studies showed that pH changes of medium due to use of bicarbonates prevented or stimulated the mycelial growth of fungi (Punja and Grogan 1982; Palmer et al., 1997). *S. rolfsii* showed optimal mycelial growth and sclerotial germination at low pH (3.0-5.5), while no growth occur above pH (8.0). Preventive effect of potassium bicarbonate on mycelial growth of soil pathogens may be partially explained by pH changes, which became more alkaline as bicarbonate concentration increased. Our result showed that *S. sclerotiorum* could not grow at pH 8.3 (at 100mM $KHCO_3$), and *R. solani* could not grow at pH 8.4 (at 750mM $KHCO_3$). Increasing concentrations of $KHCO_3$ caused growth reduction of both fungal strains. Similar inhibition effects were observed for *B. cinerea* when exposed to 20 mM of $KHCO_3$ (Palmer et al., 1997). Bicarbonates may also affect membrane permeability and change physiological processes such as oxidative phosphorylation (Olivier et al., 1998).

In the previous studies, effects of bicarbonate on both sclerotia germination and de novo sclerotia formation were investigated (Punja and Grogan 1982; Ordonez et al., 2009). Germination of *S. rolfsii* sclerotia were found to be inhibited when exposed to water agar enriched with 50 mM NH_4HCO_3 (Punja and Grogan 1982). In another study, sclerotium germination of *S. sclerotiorum* showed 18% decrease with 50 mM $KHCO_3$. Also, increasing concentrations of bicarbonate resulted in limited de novo sclerotium formation, which was strongly inhibited when $KHCO_3$ was applied at 50 mM (Ordonez et al., 2009). These findings confirmed our results which both sclerotia germination and *de novo* sclerotia formation significantly inhibited increasing concentrations of bicarbonate.

As a result, our results indicated that application of $KHCO_3$ effectively inhibited *R. solani* AG 4 HG-I and *S. sclerotiorum* which are the major fungal pathogens of bean plants. This study points out the possibility of using potassium bicarbonate controlling soil pathogens. But, further studies are needed to assess the effectiveness of $KHCO_3$ against pathogens in agricultural soils inoculated with antagonist fungi. In addition, for effective application of bicarbonate or other salts, there is a need to understand the chemical and biochemical basis of pathogen-plant-salt interactions, and to determine the effect of salts on plant tissues, since some salts including aluminium chloride and potassium phosphate are known to induce systemic resistance or plant defences including the production of phytoalexins (Mucharromah and Kuc 1991; Mecteau et al., 2002).

REFERENCES

Abdullah MT, Ali NY, Suleman P (2008). Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. Crop Protection. 27: 1354-1359.

Aharoni Y, Fallik E, Copel A, Gil M, Grinberg S, Klein JD (1997). Sodium bicarbonate reduces postharvest decay development on melons. Postharvest Biology and Technology. 10: 201-206.

Arslan U, Kadir I, Vardar C, Karabulut OA (2009). Evaluation of antifungal activity of food additives against soilborne phytopathogenic fungi. World J. Microbiol. Biotechnol. 25: 537-543.

Bae YS, Knudsen GR (2007). Effect of sclerotial distribution pattern of *Sclerotinia sclerotiorum* on biocontrol efficacy of *Trichoderma harzianum*. Appl. Soil Ecol. 35: 21–24.

Benítez T, Rincón AM, Limón MC, Codón AC (2004). Biocontrol mechanisms of *Trichoderma* strains. Inter. Microbiol. 7: 249–260.

Ben-Yephet Y, Genizi A, Siti E (1993). Sclerotial survival and apothecial production by *Sclerotinia sclerotiorum* following outbreaks of lettuce drop. Phytopathol. 83: 509-513.

Boland GJ (1990). Biological control of plant diseases with fungal antagonists: challenges and opportunities. Can. J. Plant Pathol. 12: 295-299.

Bolton MD, Thomma BPHJ, Nelson BD (2006). *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Mol. Plant Pathol. 7: 1-16.

Bombelli EC, Wright ER (2006). Efecto del bicarbonato de potasio sobre la calidad del tomate y acción sobre *Botrytis cinerea* en postcosecha (in Spanish). Cienc Invas. Agric. 33: 197-203.

Cebi M (2009). Determination of genetic variation on multinucleate *Rhizoctonia* spp. isolates from common bean plants and their rhizosphere region in black sea coastal line. Ondokuz Mayis University. Natural Sciences Institutes., Ph.D.Thesis, p.126.

Chet I, Baker R (1981). Isolation and Biocontrol Potential of *Trichoderma hamatum* from Soil Naturally Suppressive to *Rhizoctonia solani*. Phytopathol. 71: 286- 290.

Dillard HR, Cobb AC (1995). Relationship between leaf injury and colonization of cabbage by *Sclerotinia sclerotiorum*. Crop Protection, 14(8): 677- 682.

Elad Y, Chet I, Boyle P, Henis Y (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*—Scanning Electron Microscopy and Fluorescence Microscopy. Phytopathology, 73: 85-88.

Erper I, Karaca G, Ozkoc I (2011). Identification and Pathogenicity of *Rhizoctonia* Species Isolated from Bean and Soybean Plants in Samsun, Turkey. Arch. Phytopathol. Plant Protect. 44(1): 78 - 84.

Eziashi EI, Omamor IB, Odigie EE (2007). Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. Afr. J. Biotechnol. 6: 388-392.

Galindo JJ, Abawi GS, Thurston HD, Galvez G (1983). Source of inoculum and development of bean web blight in Costa Rica. Plant Disease, 67: 1016-1021.

Jamar L, Lefrancq B, Lateur M (2007). Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment. J. Plant Dis. Protect. 114: 221–227.

Karabulut OA, Smilanick JL, Gabler FM, Mansour M, Droby S (2003). Near-harvest applications of *Metschnikowia fructicola*, ethanol, and sodium bicarbonate to control postharvest diseases of grape in central California. Plant Disease, 87: 1384–1389.

Karaca GH, OÖzkoç I, Erper I (2002). Determination of the anastomosis grouping and virulence of *Rhizoctonia solani* Kühn isolates associated with bean plants grown in Samsun/Turkey. Pak. J. Biol. Sci. 5 (4): 434- 437.

Kuramae EE, Buzeto AL, Ciampi MB, Souza NL (2003). Identification of *Rhizoctonia solani* AG 1-IB in Lettuce, AG 4 HG-I in Tomato and Melon, and AG 4 HG-III in Broccoli and Spinach, in Brazil. Eur. J. Plant Pathol. 109(4): 391-395.

Mecteau MR, Arul J, Tweddell RJ (2002). Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a causal agent of potato dry rot. Mycological Research, 106(6): 688- 696.

Mills AAS, Platt HW, Hurta RAR (2004). Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. Postharvest Biology and Technology, 34: 341- 350.

Mucharromah E, Kuc J (1991). Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Protection, 10: 265-270.

Olivier C, Halseth DE, Mizubuti SG, Loria R (1998). Post-harvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Dis. 82: 213- 217.

Ordonez C, Alarcón A, Ferrera R, Hernández LV (2009). *In vitro* antifungal effects of potassium bicarbonate on *Trichoderma* sp. and *Sclerotinia sclerotiorum*. Mycoscience, 50: 380–387.

Ozkoc I, Karaca GH, Erper I (2002). Pathogenicity of *Rhizoctonia repens* Bernard on different plants and its effect on the suppression of root-rot on cucumber plants. Proc. 2nd Balkan Symp. on Veg. and Potatoes, Eds. G. Paroussi et al., Acta Horticulturae, 579: 463- 467.

Palmer CL, Horst RK, Langhans RW (1997). Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. Plant Disease, 81: 1432-1438.

Palou L, Smilanick JL, Usall J, Vinas I (2001). Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Disease, 85: 371-376.

Punja ZK, Gaye MM (1993). Influence of postharvest handling practices and dip treatments on development of black root-rot on fresh-market carrots. Plant Disease, 77: 989–995.

Punja ZK, Grogan G (1982). Effects of inorganic salts, carbonatebicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotiorum rolfsii*. Phytopathology, 72: 635- 639.

Ricker MD, Punja Z (1991). Influence of Fungicide and Chemical Salt Dip Treatments on Crater Rot Caused by *Rhizoctonia carotae* in Long-Term Storage. Plant Disease, 75: 470- 474.

Smilanick JL, Margosan DA, Mlikota F, Usall J, Michael IF (1999). Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Disease, 83: 139-145.

Sneh B, Burpee L, Ogoshi A (1991). Identification of Rhizoctonia Species. APS Press, St. Paul, Minnesota, p. 133.

Van Bruggen AHC, Whalen CH, Arneson PA (1986). Effects of inoculum level of *Rhizoctonia solani* on emergence, plant development, and yield of dry beans. Phytopathology, 76: 869- 873.

Villajuan-Abgona R, Kageyama K, Hyakumachi M (1996). Biological control of *Rhizoctonia* damping-off of cucumber by non-pathogenic binucleate *Rhizoctonia*. Euro. J. Plant Pathol. 102: 227- 235.

Wu BM, Subbarao KV (2006). Analyses of lettuce drop incidence and population structure of *Sclerotinia sclerotiorum* and *S. minor*. Phytopathol. 96: 1322-1329.

Yangui T, Rhouma A, Triki MA, Gargouri K, Bouzid J (2008). Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Protection, 27: 189-197.