

EFFECT OF SOME SALTS ON THE MYCELIAL GROWTH AND SPORE GERMINATION OF FUNGI CAUSED FRUIT ROT OF SWEET PEPPER POST-HARVEST DISEASES PATHOGENS

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ABSTRACT

*The paper aimed to inhibit evaluate the effects of some salts as natural products on postharvest diseases of sweet pepper fruits under the laboratory conditions and evaluate their effects on the development and suppression of these diseases. To achieve these aims, four different salts i.e. sodium carbonate, sodium bicarbonate, potassium nitrate and calcium chloride was applied at four concentrations, 1, 2, 3 and 4% (w/v). Salt solutions were tested on the mycelial growth and spore germination of *Ulocladium chartarum*, *Aspergillus niger*, *Fusarium semitectum* and *Geotrichum candidum*. Seven days post incubation, mycelial growth of the tested fungi were completely inhibited by the used salt solutions. Generally, the obtained results indicate a clear impact for most used salts solutions especially sodium salts.*

*Finally, the optimum concentrations of salts under the experiments study conditions for inhibiting of growth of *Ulocladium chartarum*, *Aspergillus niger*, *Fusarium semitectum*, and its spores. *Geotrichum candidum* were 4, 3, 3, 3% (w/v) for sodium carbonate, sodium bicarbonate, potassium nitrate and calcium chloride, respectively.*

***Conclusively**, the optimum concentrations of salts in this study for inhibiting growth of some fungi were 4, 3, 3, 3% (w/v) for sodium carbonate, sodium bicarbonate, potassium nitrate and calcium chloride, respectively.*

Keywords: Mycelial growth; Spore germination; salts; sweet peppers, fruits rot.

INTRODUCTION

The sweet pepper (*Capsicum annuum* L.) is considered an important vegetable crop worldwide. It is a vegetable crop belonging to the family

Solanaceae and genus *Capsicum* (Berke, 2002). Pepper contains phytochemicals that support in the protection of many diseases *e.g.*, cancer, stroke and others when eaten in diets (Ademoyegun *et al.*, 2011). It is essential for food, medicinal and industrial crops (Ashilenje, 2013). The pepper extract also used as a botanical pesticide for controlling insect and diseases of economic crops within organic agriculture systems. It is used in pickles, for flavoring sauces and in canned products. They are also used for confectionery products like bread, meat pie, burger (Ekhuemelo *et al.*, 2018). It can be consumed in many colors, is rich in both hydrophilic antioxidants *e.g.*, vitamin C, lipophilic ones *e.g.*, carotenoids or/and vitamin E (Ilić *et al.*, 2012) and with potential health-promoting properties (Bae *et al.*, 2012). Additionally, it has a high content of ascorbic acid compared to other vegetables and fruits. After the maturity stage of pepper, the major limiting factor is its relatively short shelf life (one to two weeks) which requires the use of air rather than sea transport for the export of peppers to lucrative distant markets (Maalekuu *et al.*, 2003).

Many salts were applied to inhibit fungi growth, *i.e.* sodium bicarbonate, sodium benzoate, sodium metabisulphite, and potassium metabisulphite...*etc.* Sodium bicarbonate (SBC). It can suppress postharvest anthracnose disease of papaya. Sodium bicarbonate at various concentrations (0, 1, 1.5, 2, 2.5, and 3% w/v) was used as treatment against mycelial growth and spore germination of *Colletotrichum gloeosporioides*. One week post incubation, mycelial growth was completely inhibited by SBC 3%, which was statistically similar with 2 and 2.5% SBC (99.5 and 96.5% inhibition, respectively) (Hasan *et al.*, 2012). Bicarbonate and carbonate salts of sodium and potassium have been shown to inhibit fungal pathogens of fruits, field crops, vegetables and ornamentals (DePasquale *et al.* 1990; Ziv and Zitter 1992; Punja and Gaye 1982; Aharoni *et al.* 1997; Palmer *et al.* 1997; Campanella *et al.* 2002; Palou *et al.* 2002; Arslan *et al.* 2009; Erper *et al.* 2011; Latifa *et al.* 2011).

In a related context, Ajith and Lakshmidēvi (2011) illustrated that using sodium benzoate, sodium metabisulphite and potassium metabisulphite at 1mg ml⁻¹ concentration completely inhibited the conidial germination and mycelial growth whereas, potassium nitrate inhibited the conidial germination and mycelial growth by 70 and 12% respectively. Another salt such as ammonium chloride, ammonium tartrate, calcium chloride dehydrates, potassium dihydrogen phosphate, potassium iodide and sodium sulphate increased sporulation of *H. solani* at the concentration of 0.1 M. Sodium benzoate was also the most effective compound in spores germination inhibiting (100%) for both fungi, followed by

potassium iodide (93%) for *F. solani* and ammonium acetate (88%) for *H. solani*., respectively.

Other salts significantly enhanced the mycelial growth of the fungus as potassium acetate, potassium chloride, potassium nitrate, potassium phosphate dibasic, sodium chloride, sodium sulfate and trisodium phosphate while diammonium phosphate had no significant effect (Turkkan, 2013). Moreover, El-Mougy & Abdel-Kader (2009) indicated that the application of sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity by increasing their concentrations. Their most effective concentration was 30 mg/ml reduced the disease incidence by 50 and 62.4%, respectively (El-Mougy & Abdel-Kader, 2009). Considerable interest in the use of sodium bicarbonate (NaHCO_3) and potassium bicarbonate (KHCO_3) for controlling various fungal diseases of plants (Karabulut *et al.*, 2003; Smilanick *et al.*, 2006). Lindsay (1985) indicated that bicarbonates are widely used in the food industry also, Ziv and Zitter (1992) noticed that it was effective in controlling several fungal diseases in cucumber plants. Spraying plants with NaHCO_3 solution provided good control of several plant diseases (Horst *et al.*, 1992; Arimoto *et al.*, 1997; Palmer *et al.*, 1997; Janisiewicz and Peterson, 2005). Additionally, the use of KHCO_3 -spraying solution provided the most effective protection against plant diseases (Smilanick *et al.*, 1999; Smilanick *et al.*, 2006). Sodium and/or potassium bicarbonate combined with oil were effective in controlling plant diseases (Horst *et al.*, 1992; Ziv and Zitter 1992). Calcium chloride (CaCl_2) suppressed the growth of the citrus mold pathogen *Penicillium digitatum* (Droby *et al.*, 1997). Also, calcium chloride effectively reduced silver scurf lesions on potato tubers, but not sporulation of *Helminthosporium solani*. It is known that the addition of calcium chloride can also improve the activity of biocontrol agents (McLaughlin *et al.*, 1990; Droby *et al.*, 2003).

Therefore, the objectives of this study were to evaluate the effects of some salts as natural products *in vitro* against postharvest diseases of sweet pepper fruits and evaluate their effects on the development and suppression of these diseases.

MATERIALS AND METHODS

1 Plant materials:

One hundred sweet pepper fruits were random collected from local markets of four cities at Elsharquia governorate as following: Abou Hammad, Belibes, El Zagazig and Elashir min Ramadan during the two successful seasons of 2016/2017 and 2017/2018. The collected sweet pepper fruits were kept in

paper bag and transferred into Plant Pathology Laboratory, Faculty of Technology & Development, Zagazig University, Egypt. Then fruits were incubated at 25 – 28 °C for one week. The rotted fruits of sweet pepper were classified into the different groups according to the type and colour of rots. The percentage of rotted fruits due to the different causal agents was calculated and recorded.

2 Isolation and identification of the fungal organisms:

The infected portion of the pepper fruits was cut under aseptic conditions into small bits of 5 mm into a sterile dish with the aid of scissors which was flamed over a Bunsen burner flame and dipped inside methylated spirit (Fawole and Oso, 1988). The fruits pieces were sterilized within 70% ethanol then took placed centrally on Petri dishes containing solidified potato dextrose agar (PDA). Developing fungal isolates were purified using either a single spore method and/or hyphal tip technique suggested by Lilly and Barnett (1951). The purified isolated fungi were identified according to their morphological fractures using the description of Barnett and Hunter (1998). The isolated fungi were maintained on PDA slant, kept in the refrigerator at 5 – 8 °C and sub-cultured till used. The identification was confirmed at Disease Survey and Mycology Department, Plant Pathology Institute, Agricultural Research Center, Egypt.

3. Salts used:

Carbonate and bicarbonate of sodium, potassium nitrate, and calcium chloride were purchased from Merck Chemicals (Merck, Germany), Egypt as presented in Table (1).

Table (1): The used chemical salts in the experiment.

Name of salt	Chemical formula	Molecular weight
Sodium carbonate	Na ₂ CO ₃	105.99
Sodium bicarbonate	NaHCO ₃	84.01
Potassium nitrate	KNO ₃	101.10
Calcium chloride	KCl	110.98

4 Effect of salts on mycelial growth of fungi:

The tested fungi were grown on PDA un-amended (control) or amended with the tested salts at 24 °C using PDA agar disks (diameter = 5 mm) of actively growing mycelium of *F. solani* and *H. solani*, were used to inoculate the plates. For each plate, colony diameter was determined one-week post incubation.

Colony diameter was measured as the average of the longest diameter (cm) and the shortest diameter. Inhibition of mycelial growth (IMG) was calculated using the follows equation.

$$\text{IMG} = \frac{\text{control radial growth} - \text{salt amended radial growth}}{\text{control radial growth}} \times 100 \quad [\text{Eq. 1}]$$

Three replicates were used.

5 . Effect of salts on spore germination:

Spore suspensions of each pathogen (1 ml; 9×10^5 spore's ml^{-1}) were placed in micro-tubes containing 5 ml of Potato Dextrose Broth (PDB) amended with the tested salt or untreated (control). The pH of PDB varied with the used salt and was not changed unless stated otherwise. Micro-tubes were incubated at 24 °C for one day. The germination of spores was determined using hemocytometer. Spores with germ tubes at least half the length of the spore were considered as germinated. Inhibition of spore germination (ISG) was calculated with the following equation:

$$\text{ISG} = \frac{\text{control spore germination} - \text{salt amended spore germination}}{\text{control spore germination}} \times 100 \quad [\text{Eq. 2}]$$

Three replicates were used.

6 Statistical analysis:

Statistical analyses of all experimental data were done using the statistical software SAS package (SAS Institute, 2002; available online), all comparisons were first subjected to one-way analysis of variance (ANOVA).

Significant differences between treatment means were determined using Duncan's multiple range test at $P < 0.05$ as the level of the significance (Duncan, 1955).

RESULTS AND DISCUSSIONs

The obtained results present in Table (2) indicated a clear effect of tested salts on the mycelial growth compared to the control treatment. Most of the used salts significantly reduced mycelial growth of tested fungus. Salts significantly decreased mycelial growth of *Ulocladium chartarum*. Sodium carbonate at 4 % was the most effective concentration recorded the lowest value in mycelial growth of fungi, while 1% potassium nitrate was the least effective one, which about 0.933, 6.1, respectively. Also, *Aspergillus niger* was significantly affected by 4% sodium carbonate and 4% sodium bicarbonate. *Aspergillus niger* and *Geotrichum candidum* have a relatively affect than tested fungi. *Fusarium*

semitectum was affected by the high concentrations 4% calcium chloride solution. The inhibition percent of mycelial growth for most studied fungi was significantly affected by the high concentrations of the used salts. The results presented in Table (3) indicate a clear impact on % growth inhibition of fungi and it were 77.77-100%, 74.81-100%, 52.22-86.66%, 64.81-93.33% for 4% sodium carbonate concentration, 4% sodium bicarbonate concentration, 4% potassium nitrate concentration and 4% calcium chloride concentration. This increasing recorded 53.60, 61.56, 43.42, 52.77% for *Ulocladium chartarum*, 82.03, 78.88, 71.01, 66.57% for *Aspergillus niger*, 58.79, 59.63, 77.40, 82.31% for *Fusarium semitectum* and the *Geotrichum candidum* was 74.26, 72.13, 72.77, 76.66% of carbonate sodium, sodium bicarbonate, potassium nitrate and calcium chloride solutions respectively, compared to the control treatment.

Additionally, the sodium carbonate and/or sodium bicarbonate solutions impacted on inhibition percent of spore germination of tested fungi (Table 4), and the most affective fungp with concentrations of sodium salt's solutions, were *Aspergillus niger*, *Geotrichum candidum* and *Fusarium semitectum*, compared to the control treatments, respectively. Potassium nitrate solution was less effective than other salts solutions, but it effectively inhibited the spore germination of *Fusarium semitectum*. *Aspergillus niger* was significantly affected by 3%, 4% calcium chloride, while it was not affected by potassium nitrate solutions, as the effect was relatively limited (Table 5). Based on the obtained results, it can be concluded that the optimal concentration of used salts inhibited spore germination % under the experimental conditions were 4%, 3%, 3%, 3% (w/v) for sodium carbonate, sodium bicarbonate, potassium nitrate, and calcium chloride respectively. A related context, the significance of interaction was tested between fungi, salts and salts concentrations for mycelial growth, inhibition of mycelial growth, spore germination and % inhibition of spore germination of fungi. Data presented in Table 6, indicate to statistical analysis of the experiment. The interactions between all studied factors were significant, while the interaction between tested fungi, salts, salt concentrations for inhabitation of mycelial growth of fungi was not significant. these results are consistent with the results of (Arslan *et al.*, 2009), (Erper *et al.*, 2011), (Latifa *et al.*, 2011), and (Turkkan *et al.*, 2017). In line with the study, several previous studies have also confirmed carbonate and bicarbonate solutions including sodium and potassium nitrate to have inhibitory effects on the mycelial growth of different fungi (Palmer *et al.*, 1997; Palou *et al.*, 2002; Droby *et al.*, 2003; Latifa *et al.*, 2011).

Moreover, many studies demonstrated that sodium carbonate and sodium bicarbonate salts exhibited fungistatic rather fungicidal activity against many

Table (6): Effect of the used salts and its concentrations on the mycelial growth, % inhibition of mycelial growth, spore germination and % inhibition of spore germination of the studied fungi.

Treatments	Mycelial growth	Inhibition of mycelial growth (%)	Spore germination	Inhibition of spore germination (%)
Fungi (A)				
<i>Ulocladium chartarum</i>	4.072 ^a	54.741 ^d	43.208 ^a	56.791 ^d
<i>Aspergillus niger</i>	2.283 ^d	74.625 ^a	42.979 ^b	56.812 ^c
<i>Fusarium semitectum</i>	2.741 ^b	69.532 ^c	37.937 ^c	62.020 ^b
<i>Geotrichum candidum</i>	2.343 ^c	73.953 ^b	36.104 ^d	63.791 ^a
LSD (0.05)	0.0581	0.6450	0.9000	0.9990
Salts (B)				
Sodium carbonate	2.785 ^c	69.046 ^a	28.145 ^c	71.750 ^b
Sodium bicarbonate	2.872 ^b	68.074 ^b	23.854 ^d	76.145 ^a
Potassium nitrate	3.045 ^a	66.152 ^c	64.789 ^a	35.229 ^d
Calcium chloride	2.737 ^d	69.579 ^a	43.500 ^b	56.291 ^c
LSD (0.05)	0.0581	0.6450	0.9000	0.9990
Concentrations (C)				
1%	3.929 ^a	56.338 ^d	55.625 ^a	44.375 ^d
2%	3.262 ^b	63.746 ^c	44.312 ^b	55.583 ^c
3%	2.593 ^c	71.175 ^b	34.750 ^c	65.208 ^b
4%	1.656 ^d	81.592 ^a	25.541 ^d	74.250 ^a
LSD (0.05)	0.0581	0.6450	0.9000	0.9990
Interactions				
A × B	**	**	**	**
A × C	**	**	**	**
B × C	**	**	**	**
A × B × C	**	NS	**	**
LSD of interactions:				
A×B=	0.0957	1.0639	1.7470	1.8800
A×C=	0.9300	10.341	3.9180	1.1520
B×C=	1.2040	13.380	10.225	10.292
A×B×C=	1.0648	-	10.618	10.099

fungi (Punja and Grogan, 1982; DePasquale and Montville, 1990; Palou *et al.*, 2002; Latifa *et al.*, 2011). The inhibitory effect of bicarbonate salts on fungi was probably due to the reduction in fungal cell turgor pressure which resulted in the collapse and shrinkage of hyphae and spores, consequently, the inability of fungi sporulation (Fallik *et al.*, 1996). In another study, the results confirmed that a solution of potassium nitrate increased inhibition of the growth of some fungi on soybean plants and it was due to indicate that potassium accumulation in inhibitory growth sites (Sugimoto *et al.*, 2009). Türkkan *et al.* (2017) confirmed that carbonate and carbonate salts have broad-spectrum antimicrobial properties and are generally recognized as safe compounds which do not require expensive testing and validation by regulatory agencies. Therefore, they are very promising candidates for postharvest diseases, especially in fresh commodities to which the application of synthetic fungicides is banned such as sweet pepper. Additionally, our results are consistent with the results of Stošić *et al.* (2014), who confirmed that calcium chloride salt has a great effect and important role in inhibiting of the growth of some fungi.

CONCLUSION

Based on the obtained results and it confirmed a significant effect for most used salts solutions especially sodium salts. It significantly inhibited growth of the tested fungi. Also, the effective concentrations of these salt's solutions under the experiments study conditions to inhibit the growth of *Ulocladium chartarum*, *Aspergillus niger*, *Fusarium semitectum* and *Geotrichum candidum* were 4, 3, 3, 3% (w/v) for sodium carbonate, sodium bicarbonate, potassium nitrate and calcium chloride, respectively.

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تأثير بعض الأملاح على نمو الميسليوم وإنبات جراثيم الفطريات التي تصيب ثمار الفلفل الحلو ما بعد الحصاد

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هدفت هذه الدراسة إلى تقييم تأثير بعض الأملاح المعدنية في تثبيط بعض الأمراض الفطرية لثمار الفلفل الحلو في مرحلة ما بعد الحصاد تحت الظروف المعملية، وكذا تقييم أثارها على تطور أو منع الإصابة بها. لتحقيق هذه الأهداف؛ أجريت دراسة معملية استخدم فيها أربعة أملاح مختلفة هي كربونات الصوديوم وبيكربونات الصوديوم و نترات البوتاسيوم وكلوريد الكالسيوم بتركيزات مختلفة 1 و 2 و 3 و 4% (وزن / حجم) بالإضافة الي معاملات المقارنة. استخدم محاليل الأملاح كمعاملات ضد النمو الفطري وإنبات الجراثيم في فطريات، *Ulocladium chartarum* ، *Aspergillus Niger* ، *Geotrichum candidum* ، *semitectum Fusarium*.

وذلك بعد سبعة أيام من التحضين. وقد أدت المعاملة لتلك الاملاح الى تثبيط نمو الفطريات تمامًا. وبشكل عام، تشير النتائج التي تم التوصل إليها إلى وجود تأثير معنوي لمعظم م الأملاح المستخدمة، وخاصة أملاح الصوديوم، حيث كانت فعالة في تثبيط نمو معظم الفطريات وجراثيمها. كانت التركيزات 4، 3، 3، 3 % (وزن/حجم) لاملاح كربونات الصوديوم ، بيكربونات الصوديوم ، نترات البوتاسيوم، وكلوريد الكالسيوم على التوالي تحت ظروف الدراسة هي الأكثر قدرة لتثبيط نمو فطريات *Ulocladium chartarum* ، *Aspergillus Niger* ، *Fusarium semitectum* ، *Geotrichum candidum* .
التوصية: التركيزات المثلى من الأملاح المختبرة في هذه الدراسة 4، 3، 3، 3% (وزن / حجم) لأملاح كربونات الصوديوم، بيكربونات الصوديوم، نترات البوتاسيوم، وكلوريد الكالسيوم على التوالي والتي تثبيط نمو.

الكلمات الدالة: النمو الفطري. إنبات الجراثيم. الفلفل الحلو. الاملاح المعدنية.

