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# Inhibitory Effect of Certain Chemical Food Preservatives against *Cercospora* Leaf Spot Disease of Sugar Beet

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## ABSTRACT

The antifungal activity of citric acid and its salt (sodium citrate) as chemical food preservatives was evaluated against *Cercospora beticola in vitro* and *in vivo*. The inhibitory effect of both compounds was studied against mycelial growth of the pathogen *in vitro* at concentrations*, i.e.* 0, 10, 20, 30, 40 and 50 mM. Both of citric acid and sodium citrate were able to inhibit the mycelial growth of the pathogen when they were added to the medium at the tested concentrations. Citric acid at 50 mM had the highest effect on the inhibition growth of the pathogen being 83.70% and also had antifungal activity. The percentage of inhibition growth increased as the tested compounds concentration increased. Data also indicate that microscopic examination of treated plates with citrate shows that this treatment reduced the numbers of *C. beticola* spores on the medium at all tested concentrations and reached its maximum reducing at concentration 50 mM of citric acid compared to untreated plates. The results revealed that spraying of diseased plants with citric acid and sodium citrate either alone or in combination at concentration 50 mM was effective in decreasing disease severity of Cercospora leaf spot compared to untreated plants. The highest reduction in disease severity was observed in case of citric acid treatment. The results revealed that foliar application of these compounds enhanced significantly root yield and sugar percentage as compared to the control.

**Keywords:** *Cercospora beticola*, sugar beet**,** citric acid, sodium citrate, root yield, sugar percent.

## INTRODUCTION

*Cercospora* leaf spot (CLS) caused by *Cercospora beticola* is one of the most devastating diseases of sugar beet (*Beta vulgaris* L.) in all production areas (Rosenzweig *et al.,* 2015 and El-Fawy, 2016). It causes reduction in root weight and sugar content (Shane and Teng 1992). Losses of sugar percent occur as new leaves are grown to replace those heavily damaged by Cercospora leaf spot (Vereijssen *et al.*, 2003). Till now, there are no available resistant varieties for this disease (Gado, 2007).

Citric acid and its salt (sodium citrate) are called citrate. They are used as chemical preservatives to enhance the shelf life of food, because they have a high ability to inhibit a wide range of microorganisms (Thomas and Wimpenny, 1996 and Blaszyk and Holley, 1998). Citric acid is created in almost all plants (Gogoreena *et al.,* 1997) but most concentrated in lemon and lemon sour. Some mechanisms have been suggested to explain the inhibitory effect of citric acid on microorganisms. Such as a lowered pH resulting from this acid may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis (Cole and Keenan 1987 and Bracey *et al*., 1998). Other possibilities have also been proposed including the membrane disruption (Bracey *et al.,* 1998 and Stratford and Anslow 1998), the interruption of metabolic reactions (Krebs *et al.,* 1983), and the accumulation of toxic anions (Eklund 1985). Citric acid inhibits the growth of proteolytic *Clostridium botulinum* due to its $Ca^{+2}$ chelating activity (Graham and Lund 1986).

Citrate is among the intermediate organic acids in Krebs cycle which produces cellular energy by oxidative phosphorylation (Wills *et al.,* 1981). It is one of the mobile forms of iron inside the plant so it plays an important role in iron transport inside plants (Hell and Stephan, 2003). Several studies also mentioned the role of citrate in control of many plant diseases (Abdel-Monaim and Ismail, 2010 and Mohamed *et al.,* 2015). Coumaric acid, citric acid, propylgalate and salicylic acid were effective against Fusarium isolates at 100 and 200 ppm (Abdel-Monaim and Ismail, 2010). On the other hand, foliar spraying with citrate had an effective in enhancing growth plant and yield. Citric acid treatment increased plant height, yield and its components, as well as protein content in common bean, pea and faba bean (Abd- Allah *et al.,* 2007). Also, application of citric acid as a foliar spray at 200 ppm increased vegetative growth, dry weight and yield of tomato plants (Ali *et al.,* 2009). Jafari and Hadavi, (2012) he mentioned that foliar application of basil plants with citric acid at 0.1% (w/v) produced higher biomass and essential oil yield.

The aims of this study were to evaluate the effectiveness of citric acid and sodium citrate either alone or in combination for controlling Cercospora leaf spot disease of sugar beet and their effect on root yield and sugar content.

## MATERIALS AND METHODS

**Source of *Cercospora beticola* isolate:**

A pathogenic isolate of *C. beticola* previously recovered from sugar beet plants, showing typical symptoms of Cercospora leaf spot disease, was used in this study (El-Fawy, 2016).

**Effect of citric acid and sodium citrate on mycelial growth of *C. beticola*:**

These compounds were tested to study their effect on the mycelial growth of *C. beticola in vitro*. Both citric acid and sodium citrate were added to PDA medium at concentrations 0, 10, 20, 30, 40 and 50 mM and then mixed thoroughly just before solidification as mentioned by Shokri (2011). Moreover, the fungicide Cabrio Top WG 60% (Metiram + Pyraclostrobin) was used at concentrations 0, 20, 40, 60, 80 and 100 ppm as comparison treatment. The stock medium (PDA) for each concentration was poured into Petri dishes. Four plates were used for each treatment as replicates, as well as other four Petri dishes without citrates or the fungicide were served as a control. All plates were inoculated in the center with 6 mm discs of *C. beticola* and incubated at 25°C. Data were recorded as a diameter of linear growth and percentage of growth inhibition of the pathogen was calculated by using the following formula:

$$\mathbf{R = (C - B/C) \times 100}$$

**Whereas:**

**R= % of growth inhibition, C = growth in control and B = growth in treatment.**

**Effect of citric acid and its salt on the number of spores of *C. beticola*:**

Treated plates with citrate were microscopic exanimated for spores account of *C. beticola* by the microscope at 40X magnification and four microscopic fields from each treatment were examined as replicates and calculated the average of number spores/ microscopic field.

**Preparation of spore suspension of the fungal pathogen isolate:**

The spore suspension of *C. beticola* isolate was prepared by growing it on PDA medium for two weeks. After an incubation period, the growth isolate was collected and blended as mentioned by El-Fawy, (2016). The concentration of spore suspension was adjusted to $1\times10^5$ per mL of water using a hemacytometer.

**Effect of spraying with citric acid and sodium citrate either alone or in combination on Cercospora leaf spot disease of sugar beet:**

Field experiments were carried out at the Nubaria Research Station, El-Behera, Governorate, Egypt during growing seasons 2015/2016 and 2016/2017 to study the effect of citric acid, sodium citrate and their combination on Cercospora leaf spot disease. Seeds of Pleno sugar beet cultivar (multigerm seeds) obtained from Nobaryia Sugar Refining Company (NSRC) were sown directly in plots of $3\times3.5$ m$^2$ arranged in completely randomized design, with three plots for each treatment as replicates. Sixty days old plants were sprayed with *C. beticola* spore suspension with the concentration $1\times10^5$ spores/mL as mentioned by El-Fawy (2016). Infected plants were sprayed with citric acid, sodium citrate either alone or in combination at concentration 50 mM when the first sign of disease has appeared (Gado, 2007). The spreading agent Triton Mok (a commercial adhesive) was added to the spray solution to ensure full distribution on the surfaces of plants. The fungicide Cabrio Top 60% obtained from Shora Company was applied at concentration 0.50 g/L water as a control. Each treatment was applied either as one and two sprays (10 days between each one) for different plots. Control plants were sprayed with distilled water. Disease severity was calculated after each spray using the diseased scales by Jones and Windels (1991) as follows: 0= no leaf lesions, 1= 25% or less infected leaf area, 2= 26 to 50 %, 3= 51 to 75 % and 4= 76 to 100% infected leaf area. At the end of the experiment, root yield and sugar percent were determined. Sugar percent was measured at the sugar factory laboratory at Nobaryia Sugar Refining Company, using standard polarimetric method estimated by Schneider *et al*., (2002).

**Statistical analysis:**

The data were subjected to statistical analysis using the MSTAT-C (1991) program version 2.10. Least significant difference (L.S.D) was employed to test for significant difference between treatments at P=0.05 (Gomez and Gomez, 1984).

## RESULTS

**Effect of citric acid, sodium citrate and the fungicide Cabrio Top on mycelial growth of *C. beticola*:**

From Table (1) and Fig. (1) it was clear that citric acid and sodium citrate significantly (P=0.05) inhibited the mycelial growth of *C. beticola* at the tested concentrations compared to the control. As shown in Fig. (1), citric acid was the most effective in reducing the mycelial growth of *C. beticola* on PDA medium more than sodium citrate and the fungicide Cabrio Top. The highest inhibition percentage of the mycelial growth of the pathogen was occurred by citric acid at the concentration of 50 mM (83.70 %), followed by fungicide Cabrio Top (77.41%) at 100 ppm. While sodium citrate at the concentration 50 mM caused the lowest inhibition (70.37%) of mycelial growth of the pathogen.

**Table 1. Effect of different concentrations of citric acid, sodium citrate and the fungicide Cabrio Top on mycelial growth of *C. beticola***

| Treatments | Conc. | Growth inhibition % |
|---|---|---|
| Citric acid | 10 mM | 53.71 |
| | 20 mM | 63.33 |
| | 30 mM | 68.89 |
| | 40 mM | 74.44 |
| | 50 mM | 83.70 |
| | 0.00 | 0.00 |
| Sodium citrate | 10 mM | 38.89 |
| | 20 mM | 48.52 |
| | 30 mM | 57.41 |
| | 40 mM | 64.82 |
| | 50 mM | 70.37 |
| | 0.00 | 0.00 |
| Cabrio Top | 20 ppm | 53.71 |
| | 40 ppm | 58.23 |
| | 60 ppm | 66.30 |
| | 80 ppm | 70.74 |
| | 100 ppm | 77.41 |
| | 0.00 | 0.00 |
| L.S.D. at 5 % for | | |
| Treatment (T) | | 1.68 |
| Concentrations (C) | | 2.37 |
| Interaction (T×C) | | 4.11 |

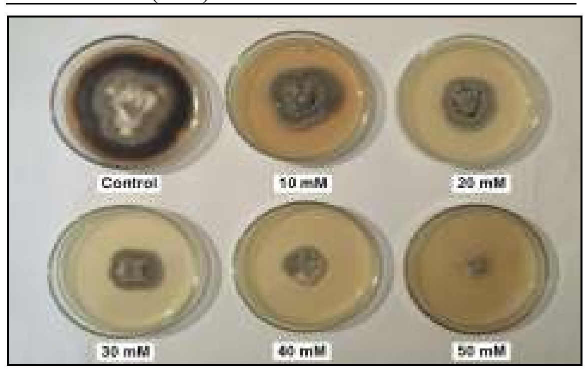


**Fig. 1. Effect of citric acid at different concentrations on mycelial growth of *C. beticola*.**

**Effect of citric acid and sodium citrate on the number of *C. beticola* spores on PDA medium:**

Results presented in Table (2) indicate that microscopic examination of treated plates with citrate shows that this treatment reduced the numbers of *C.*

*beticola* spores on the medium at all tested concentrations and reached its maximum reducing at concentration 50 mM of citric acid compared to untreated control plates (P=0.05) as explained in Fig. 2. There are significant differences among treatments in reducing the number of spores of the pathogen.

**Table 2. Effect of citric acid and its salt on the number of spores of *C. beticola*. on PDA medium**

| Treatments | Number of spores/microscopic field (at mM) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| citric acid | 26.33 | 13.00 | 9.00 | 6.33 | 4.00 | 2.00 |
| sodium citrate | 26.33 | 15.67 | 12.00 | 8.00 | 6.33 | 3.33 |
| L.S.D. at 5% | | | | | | |
| Treatments (T) | 1.88 | | | | | |
| Concentrations (C) | 3.25 | | | | | |
| Interaction(T×C) | 4.59 | | | | | |

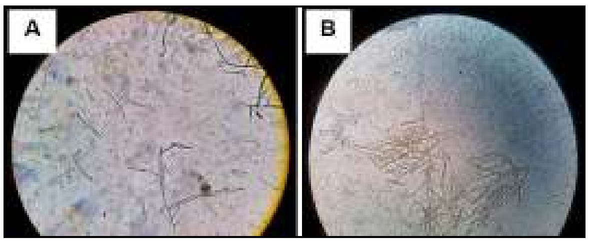


**Fig. 2. Effect of citric acid on the number of spores of *C. beticola*, microscopic field untreated (A) and microscopic field treated with citric acid (B) at 40X magnification.**

**Effect of foliar application of citric acid, sodium citrate either alone or in combination on the disease severity of Cercospora leaf spot of sugar beet:**

The application of these compounds as a foliar spray was very beneficial in reducing Cercospora leaf spot disease compared to the control. Data in Table (3) indicate that treatment with citric acid at concentration 50 mM was the highest effect on the disease (12.74 and 14.86%) at both seasons 2015/2016 and 2016/2017, respectively when it's used twice and its the most decreasing to the development of the disease as explained in Fig. 3.

**Table 3. Effect of citric acid, sodium citrate either alone or in combination as a foliar application on disease severity of Cercospora leaf spot of sugar beet under field conditions.**

| Treatments | Disease severity (%) | | | | | |
|---|---|---|---|---|---|---|
| | Season 2015/2016 | | | Season 2016/2017 | | |
| | One spray | Two sprays | Mean | One sprays | Two sprays | Mean |
| Citric acid | 13.06 | 12.74 | 12.90 | 15.14 | 14.86 | 15.00 |
| Sodium citrate | 15.17 | 14.08 | 14.63 | 15.89 | 14.93 | 15.41 |
| Synergistic action | 16.54 | 16.43 | 16.66 | 18.00 | 16.88 | 17.44 |
| Cabrio top 60% | 11.51 | 10.42 | 10.91 | 10.93 | 9.67 | 10.21 |
| Control | 42.61 | 48.16 | 45.29 | 47.18 | 50.45 | 48.82 |
| Mean | 19.71 | 20.35 | - | 21.39 | 21.36 | - |
| L.S.D. at 5% | | | | | | |
| Treatments (T) | | 2.19 | | | 2.70 | |
| Sprays (S) | | 1.39 | | | 1.71 | |
| Interaction (T×S) | | 3.10 | | | 3.82 | |

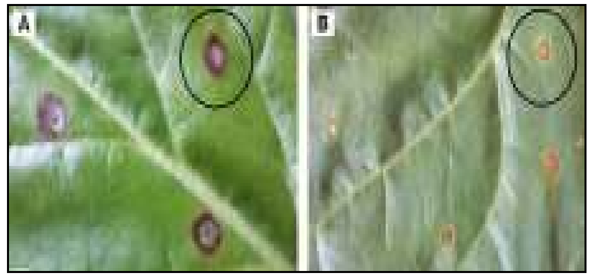


**Fig. 3. At left, untreated sugar beet leaf (A ) and at right, treated leaf with citric acid (B).**

On the other hand, Cabrio Top fungicide 60% gave the highest reduction in the disease severity at concentration 0.50 g/l (10.42 and 9.67%) at both seasons 2015/2016 and 2016/2017, respectively when it's use. The combination of citric acid and sodium citrate was the lowest effect on the disease. Treated plants twice with all treatments were the better in reducing the disease severity than the treatment once. The results show that there are significant differences among the treatments in decreasing the effectiveness of the disease severity. On the other hand, there were no significant differences between sprays on the disease at both seasons.

**Effect of foliar application of citric acid, sodium citrate either alone or in combination on root yield of sugar beet:**

The data presented in Table (4) indicate that foliar application of citric acid, sodium citrate and their combination had a significant on root yield of sugar beet crop. Data also indicated that these compounds showed a significant positive effect on the root yield of sugar beet crop. The highest root yield was recorded in citric acid treatment at concentration 50 mM at both seasons (2015/2016 and 2016/2017) being 36.17 and 34.94 tons/feddan, respectively as compared to the control (P=0.05). The fungicide Cabrio top gave the lowest values of root yield. These results showed that all treatments led to increase in root yield of the treated plants at both seasons compared to untreated control. A root yield value was reduced by increasing disease severity. There are significant differences among the treatments on root yield of sugar beet.

**Effect of citric acid, sodium citrate either alone or in combination as a foliar application on the sugar percentage in sugar beet roots:**

Data shown in Table (5) indicate that foliar spray with citric acid, sodium citrate and their combination at the concentration 50 mM significantly (P=0.05) increased the sugar percentage during both seasons. Generally, application of citric acid had more effect on sugar percentage (21.22 and 21.18%) during seasons 2015/2016 and 2016/2017 respectively, than the other treatments when compared to the control (17.15 and 16.76%). The highest sugar percentage obtained from citric acid treatment when it's applied twice. Data also revealed that there were significant differences among all treatments at both seasons. Application of all treatments at two times sprays was the best results on sugar percentage at both seasons.

**Table 4. Effect of foliar application of citric acid, sodium citrate either alone or in combination on root yield of sugar beet under field conditions**

| Treatments | Root yield (tons/feddan) | | | | | |
|---|---|---|---|---|---|---|
| | Season 2015/2016 | | | Season 2016/2017 | | |
| | One spray | Two sprays | Mean | One sprays | Two sprays | Mean |
| Citric acid | 34.11 | 36.17 | 35.14 | 34.88 | 34.94 | 34.91 |
| Sodium citrate | 31.53 | 32.06 | 31.80 | 32.14 | 33.10 | 32.62 |
| Synergistic action | 27.81 | 28.19 | 28.00 | 28.00 | 28.26 | 28.13 |
| Cabrio top 60% | 29.02 | 31.10 | 30.06 | 32.21 | 32.33 | 32.27 |
| Control | 25.08 | 25.08 | 25.08 | 26.90 | 26.90 | 26.90 |
| Mean | 29.51 | 30.52 | - | 30.83 | 31.11 | - |
| L.S.D. at 5% | | | | | | |
| Treatments (T) | | 2.82 | | | 2.62 | |
| Sprays (S) | | 1.78 | | | 1.66 | |
| Interaction (T×S) | | 3.98 | | | 3.70 | |

**Table 5. Effect of foliar application of citric acid, sodium citrate either alone or in combination on sugar percentage of sugar beet under field conditions**

| Treatments | Sugar % | | | | | |
|---|---|---|---|---|---|---|
| | Season 2015/2016 | | | Season 2016/2017 | | |
| | One spray | Two sprays | Mean | One sprays | Two sprays | Mean |
| Citric acid | 20.07 | 21.22 | 20.65 | 21.18 | 21.06 | 21.48 |
| Sodium citrate | 19.63 | 19.54 | 19.59 | 18.92 | 19.14 | 19.03 |
| Synergistic action | 19.89 | 20.03 | 19.96 | 19.63 | 19.81 | 19.72 |
| Cabrio top 60% | 18.91 | 19.18 | 19.05 | 18.74 | 19.11 | 18.93 |
| Control | 17.15 | 17.15 | 17.15 | 16.76 | 16.76 | 16.76 |
| Mean | 19.13 | 19.42 | - | 19.19 | 19.18 | - |
| L.S.D. at 5% | | | | | | |
| Treatments (T) | | 0.63 | | | 0.37 | |
| Sprays (S) | | 0.40 | | | 0.23 | |
| Interaction (T×S) | | 0.90 | | | 0.52 | |

## DISCUSSION

Cercospora leaf spot is an economically important disease in all the sugar beet production areas (El-Fawy, 2016 and Piszczek *et al.,* 2017). The obtained results demonstrate that citric acid and sodium citrate can inhibit significantly the growth of the pathogen at the tested concentrations. These results are similar to those obtained by Thomas and Wimpenny (1996) who showed that lactic and citric acids inhibit the growth of *Arcobacter butzleri*. This study revealed that citric acid was the highest effect in reducing the mycelial growth of *C. beticola* when it's added to the medium at concentration 50 mM. Citrate could kill bacteria in many ways. The frist way it could chelate away specific metals from bacteria and thus stop some enzymes from working as reported by Graham and Lund (1986) who found that citric acid inhibits the growth of proteolytic *Clostridium botulinum* due to its $Ca^{2+}$ chelating activity (Graham and Lund 1986). The second way, it could change pH drastically depending on the concentration (Cole and Keenan 1987 and Bracey *et al.,* 1998) and the third way, citric acid is a part of the Krebs cycle (Wills *et al.,* 1981). Data also indicate that treated plates with citrate contained a few number of spores of *C. beticola* where this number was reduced by increasing the concentration of citrate in the medium**.** Citric acid was the most effective in reducing the number spores of *C. beticola*. These results are in conformity with those obtained by Aderiye *et al.,* (1998) who reported that citric acid exhibited a higher killing rate of 0.26 CFU/h and was more effective against the germination of *Botryodiplodia theobromae* spores.

The application of citric acid and sodium citrate either alone or in combination at 50 mM as a foliar spray was very beneficial in reducing the disease severity of Cercospora leaf spot of sugar beet. Data also indicate that treatment with citric acid was more effective against the pathogen and gave the highest reduction in the disease severity compared to the control. This result is in agreement with those obtained by Mohamed *et al.,* (2015) who reported that citric acid at 2% as foliar spray was the most effective treatment against the fungal decay of artificially inoculated snap bean pods with *Botrytis cinerea* as well as naturally infected pods. In this study, the fungicide Cabrio Top was the most effective on the disease at concentration 0.50 g/L at both seasons because it had a high ability to control the disease. Organic acids generally such as citric acid reduce cytoplasmic pH and stop metabolic activities and caused the death by the susceptible organisms act on the plasmic membrane by neutralizing its electrochemical potential and increasing its permeability (Dalie *et al*, 2010). Several studies showed that citric acid and its salts such as sodium citrate inhibit the growth of the most common bacterial pathogens such as *Arcobacter spp*., *Campylobacter spp.*, Lactobacilli, *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Atabay and Corry, 1997 and Blaszyk and Holley, 1998).

The results also revealed that foliar spray with these compounds at 50 mM increased root yield and sugar percent of sugar beet crop during both seasons. Generally, application of citric acid had a higher effect on root yield and sugar percent when compared to the control. A similar effect reported by Nour *et al.,* (2012) who reported that application of citric acid at 0.5% gave the highest values of snap bean yield followed by citric acid at 0.25%. Jafari and Hadavi (2012) reported that basil plants treated with citric acid at concentration 0.1% (w/v) produced higher biomass and essential oil yield. Citric acid at 0.1% (w/v) as a foliar spray increased the vase life of cut tuberose plants and increased the size of bulblets in a synergism with foliar Fe (Eidyan *et al.,* 2014). In another study, Talebi *et al.,* (2014) found that application of citric acid at 100 and 300 mg/L and malic acid at 300 mg/L significantly increased the fresh weight of root plants. Also, the plant height and peduncle length were significantly increased in all applied levels of citric acid and malic acid. Citrate is among the intermediate organic acids in Krebs cycle which produces cellular energy by oxidative phosphorylation (Wills *et al.,* 1981). The increase in root yield and sugar percent may be due to the role of the citrate in promoting plant growth, thereby enhancing root yield and sugar percent. The same results were also obtained by Maleki *et al*. (2013) reported that citric acid significantly increased shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of sweet basil. and Potassium citrate at 2.5 g/L and salicylic acid at 200 ppm caused enhancement of growth and yield characters of cotton plants under salt conditions (El-Beltagi *et al.,* 2017). From these results, it can be concluded that application of citric acid and its sodium salt as a foliar spray led to reduce the disease severity of Cercospora leaf spot of sugar beet and increase the root yield and sugar percent. Moreover, these compounds did not appear any toxicity to the plants.

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## تقييم التأثير التثبيطي لبعض المواد الحافظة للأغذية ضد مسبب مرض تبقع الأوراق السركسبورى فى بنجر السكر

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تم تقييم حامض الستريك وملحه الصوديومى (سترات الصوديوم) كمواد حافظة للأغذية ضد الفطرسركسبورا بيتيكولا المسبب المرضى لتبقع الأوراق السركسبورى على بنجر السكر وذلك تحت ظروف المعمل والحقل. تحت الظروف المعملية, تمت دراسة التأثير التثبيطي لكلا المركبين ضد النمو الميسليومى للمسبب المرضى على بيئة آجار البطاطس والدكستروز بتركيزات مختلفة وهى ١٠،٠ ، ٢٠ ، ٣٠ ، ٤٠ و ٥٠ ملليمول. وأظهرت النتائج أن كلا من حامض الستريك وسترات الصوديوم كانا قادرين على تثبيط النمو الميسليومى للمسبب المرضى عند إضافتهما إلى البيئة بالتركيزات المختبرة. حيث كان لحمض الستريك عند التركيز ٥٠ ملليمول تأثير قوي على تثبيط نمو المسبب المرضى بنسبة ٨٣.٧٠ ٪ وكان له نشاط قوى مضاد للفطر. وتبين أيضا أن نسبة التثبيط فى النمو زادت مع زيادة التركيز فى البيئة.وتشير البيانات أيضًا إلى أن الفحص المجهري لأطباق بترى المعاملة بكلا من حامض الستريك وسترات الصوديوم يظهر أن هذه المواد قللت من أعداد الجراثيم للمسبب المرضى على البيئة عند جميع التركيزات المختبرة ، وكان أقل ما يكون عند تركيز ٥٠ ملليمول من حمض الستريك مقارنة بأطباق بترى الغير معاملة. وأوضحت النتائج الحقلية أن نباتات البنجر المصابة بالتبقع السركسبورى بكلا من حمض الستريك وسترات الصوديوم وخليط منهما بتركيز ٥٠ ملليمول كان فعالا في تقليل شدة الإصابة بالمرض مقارنة بالنباتات الغير معاملة. ولوحظ أن أعلى انخفاض في شدة المرض كان في حالة المعاملة بحامض الستريك. أوضحت النتائج أيضا أن إستخدام هذه المركبات كمعاملة للمجموع الخضرى أدى إلى زيادة محصول الجذور ونسبة السكر فى النباتات المعاملة مقارنة بالكنترول.