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# Upgrading the Potential of Biopesticide from Actinobacteria for Controlling Onion Bacterial Rots by Mixing two Identified *Streptomyces* species in Egypt

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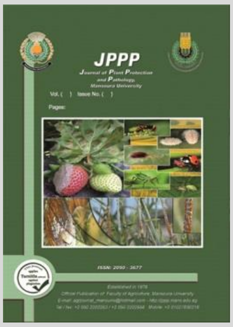


## ABSTRACT

Bacterial rots of onion are considered from the most dangerous stored and heaped onion crop diseases that cause huge loss in bulbs. Many bacterial species can induce onion bulb rots, with predominance of *Pectobacterium carotovorum* and *Burkholderia cepacia* that were used in this study. This research was conducted as an approach for improving the potential of biopesticide from actinobacteria in controlling onion bacterial rots by mixing *Streptomyces coelicolor* and *Streptomyces lavendulae* in one treatment against both *P. carotovorum* and *B. cepacia* each separately. The highest decrease in disease incidence was recorded in the mixed treatment of *S. coelicolor* and *S. lavendulae* that was recorded as 88.8% and 86.3% in case of *P. carotovorum* and *B. cepacia*, respectively, followed by the single treatment of *S. lavendulae* then the single treatment of *S. coelicolor*. Application of *S. coelicolor* and *S. lavendulae*, singly or in mixture had significantly increased the total phenols in onion bulbs but the highest increase was recorded in the mixed application treatment that recorded as 356.1% and 353.8% in case of *P. carotovorum* and *B. cepacia*, respectively. Also, the antioxidant enzymes (POX and PPO) activity was observed to be highest in case of the mixed treatment against both *P. carotovorum* and *B. cepacia*. On the otherhand lipid peroxidation was significantly decreased in all treatments but the highest decrease percentage was detected in the mixed treatment which was 53.3% and 57.7% in case of *P. carotovorum* and *B. cepacia*, respectively. Based on their efficiency and eco-safety, *S. coelicolor* and *S. lavendulae* have been recommended to be mixed in one treatment to increase the potential of biopesticide used for controlling onion bacterial rots.

**Keywords:** Disease incidence, Onion, bacterial rots, *Pectobacterium carotovorum, Burkholderia cepacia, Streptomyces coelicolor* and *Streptomyces lavendulae.*

## INTRODUCTION

In Egypt, onion (*Allium cepa* L.) is an important domestic and export crop. Onions are ranked third in export after citrus and potatoes. The annual cultivated area in Egypt is about 195000 feddans, with a total production of 209 million tons, sufficient for local consumption and export, and processed surplus of approximately one million tons is achieved, along with fresh export to abroad (FAO, 2019). Egypt ranks the third in terms of productivity in the world after China and India. Onions have a high nutritional value for humans, as they contain many important salts and nutrients, in addition to the abundance of vitamins as well as antioxidants, which makes it a strong and effective component for human health, as well as, onion decrease the level of sugar in the human blood.

Onion plants are attacked with many diseases; fungal, viral, bacterial and nematodes (Abdalla *et al.* 2019) that cause a huge loss in the productivity, storability and export. Onion bacterial rots are considered from the most dangerous bulbs storage diseases that perhaps classified into at least three different diseases; sour skin (caused by *Burkholderia cepacia*), slippery skin (caused by *Burkholderia gladioli* subsp. *allicola*) and soft rot (commonly caused by *Pectobacterium carotovorum*) (Chaput, 1995; McNab; 2004 and Mansour *et al.* 2011). Onion rotting bacteria are soil borne pathogens, but their symptoms appear on onion bulbs in stored and/or transported crop when surrounding by favorable environmental conditions such as high humidity and warm ambient temperature. The symptoms of bacterial rots include watery rot of the bulb fleshy scales which turn brown and the rotten bulbs have a bad odor (Mansour *et al.*, 2011; Badr and El-Sharkay, 2018). Yield losses in onion crop between 5 to 50 % were recorded due to the bacterial rots (Schwartz and Gent, 2004) therefore, it is too extremely important to achieve an effective and safe control measures.

Actinobacteria are gram-positive bacteria, produce a lot of metabolites with biological activity and high commercial values such as vitamins, antibiotics, alkaloids, plant growth promoters, enzymes and enzyme inhibitors, so they are being considered excellent biocontol agents against plant pathogens (Chaursia *et al.*, 2018; Badr and El-Sharkawy, 2018 and El-Sharkawy *et al.*, 2018a). They have different mechanisms, direct effect, including direct parasitism, antimicrobial compounds production, nutrients and space competition (Ara *et al.*, 2012 and Badr and El-Sharkawy 2018) and indirect effect through inducing the plant resistance by improvement the biochemical and physiological responses of the plant and strengthening the cell walls (Shafie *et al.*, 2016; Awla *et al.*, 2017; Badr and

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El-Sharkawy 2018 and El-Sharkawy *et al*., 2018a). Actinobacteria are not only effective in controlling plant pathogens but also act as a good plant growth promoters through helping plants to mobilize and get nutrients (Gopalakrishnan *et al.*, 2014 and El-Sharkawy *et al*., 2018a).

The aim of this study is; 1) evaluating the idea of mixed application of *S. coelicolor* and *S. lavendulae* in controlling onion bacterial rots under storage conditions, and 2) estimating some defensive parameters in the onion bulbs as a response to the treatment with such biocontrol agents and their mixture.

# MATERIALS AND METHODS

The present study was conducted during 2019 growing seasons. Domestic onion (Ahmar bred cultivar) was grown in a private farm located in Aga, Dakahlia Governorate, Egypt.

**Plant material:**

Domestic onion transplants (L.) cv. Ahmar bred cultivar was kindly gifted by the Horticulture Research Institute, ARC, Egypt.

**Bacterial inocula:**

In this research two strains (*Pectobacterium carotovorum* and *Burkholderia cepaci)* of onion bacterial rot pathogens were used which were previously isolated and identified by Badr (2011) and stored in frozen glycerol (-20$^{o}$ C) and were recharacterized before use.

**Bio-Control Agents (BCAs):**

Two isolates of actinobacteria; *Streptomyces coelicolor* ($10^6$ spore/ ml) and *S. lavendulae* ($10^6$ spore/ ml) were used in this study, which were previously isolated, identified and tested for antagonistic potentials *in vitro* against *Burkholderia cepacia* and *Pectobacterium carotovorum* in a study by Badr (2011).

*S. coelicolor* and *S. lavendulae* were tested *in vitro* for the antagonism between each other using perpendicular method (Oskay *et al.*, 2004).

***In vivo* studies:**

A Pot experiment was conducted in Dakahlia governorate, Egypt during 2019, to estimate the impact of using each of *S. coelicolor* and *S. lavendulae* in one combination for controlling the bacterial rots of onion. Plastic pots containing clay: sandy soil (2:1) were used to cultivate onion transplants (Ahmar bred cultivar), two transplants/pot, the pots were divided into two sets; the first one for studying the suggested treatments against *Pectobacterium carotovorum* while the second was for *Burkholderia cepaci*. The tested treatments were as follow: 1) *S. coelicolor*, 2) *S. lavendulae*, 3) *S. coelicolor* mixed with *S. lavendulae* and 4) control, all of them were added to the soil before onion transplanting. Six replicates were done for each treatment. One month before harvest, the soil was inoculated by the bacterial pathogens as suspension inoculum (with $10^6$ CFU/ml) each in its set. After 4 months from planting, the onion was harvested then kept two weeks in open air before being stored for 8 months.

**Postharvest examinations**

**Disease assessment:**

By the end of storage period, the disease incidence of bacterial rots was determined in onion bulbs for each treatment according to the method followed by Badr (2011) using the following formula:

**DI (%) = (Number of diseased bulbs / total number of stored bulbs) × 100.**

**Biochemical determinations**

Immediately after yield harvesting, the activities of some defense-related enzymes (polyphenol oxidase and peroxidase) were assessed in onion bulbs. Extraction and determination of polyphenol oxidase (PPO) and peroxidase (POD) enzymes were performed according to Maria *et al.* (1981) and Maxwell and Bateman (1967) respectively. The total phenol content was estimated using folin ciocalteau reagent according to Malik and Singh (1980). On the other hand, lipid peroxidation was estimated in the form of thiobarbituricacid reacting substances (TBARS) by the method of Shao *et al.* (2005) where the malondialdehdyde content (MDA) was estimated and calculated asμmol/gram fresh weight.

**Statistical analysis:**

Data were statistically analyzed by CoStat system (CoHort Software, U.S.A) version 6.4 (CoStat 2005). The means comparison was done using Duncan's multiple range test (Duncan 1995) at $P \leq 0.05$. Different letters indicates significant difference between means.

# RESULTS AND DISCUSSION

**Evaluating the *in vitr*o antagonism between *S. coelicolor* and *S. lavendulae***

Studying the *in vitro* antagonism between *S. coelicolor* and *S. lavendulae* by the perpendicular method showed that there is no antagonism between them, so they can be used in a mixture for biological control.

**Effect of BCAs on the reduction percentage in the diseases incidence of onion bacterial rots**

Data presented in Fig. 1 (A & B) indicated that all the tested treatments significantly decreased the incidence of onion bacterial rots disease, but the highest decrease was observed with the application of *S. coelicolor* and *S. lavendulae* combination in case of *P. carotovorum* and *B. cepacia* that recorded as 88.8.0% and 86.3%, respectively, followed by *S. lavendulae* treatment (69.5%, 65.7) and then *S. coelicolor* treatment (66.1, 64.8%).The obtained results are in concord with that of Abdallah *et al*. (2013), who noticed that the application of *S. coelicolor* caused significant decrease in onion bacterial rots produced by *P. carotovorum* and *B. cepacia*, Badr and El-Sharkawy (2018) who found that the use of *S. coelicolor* and Arbuscular mycorrhizal fungi (AMF) individually or in mixture resulted in a significant reduction in the disease incidence of onion bacterial rots, and Mansour *et al*. (2008) who found that the use of *Streptomyces sioyaensis* caused significant decrease in potato sot rot disease incidence caused by *pectobacterium carotovorum.*

Actinobacteria have several properties that explain its role in plant health and protection against pathogens from which 1) the capability to colonize plant surface, 2) antibiosis against phytopathogens and 3) degradation of phytotoxins (Doumbou *et al*, 2001). Actinobacteria also play an important role in inducing plant defense against pathogens through different mechanisms; eg: inducing the activity of some enzymes related to the defense in plant, phenolic compounds accumulation in plant which play an important role in plant diseases resistance because of their toxicity nature and their incorporation in the synthesis of lignin and subrin the matter that lead to increasing the thickness of the cell wall so act as physical barrier against pathogen invasion (Abdalla *et al*., 2011; Li *et al*., 2017; Farouk *et al*., 2017 and Badr and El-Sharkawy 2018).

Beside its effective role as BCAs, actinobacteria also act as good plant growth promoters through solubilizing soil minerals to be available for plant uptake and use or by supplying the plant with compounds from its own production like phytohormones and siderophores (Doumbou *et al*., 2001). In particular, *S. coelicolor* was proven to produce the plant hormone indole-3-acetic acid (Manulis *et al*., 1994) and also was established to produce siderophores that facilitate iron uptake by plant (Lautru *et al*., 2005 and Badr, 2011).

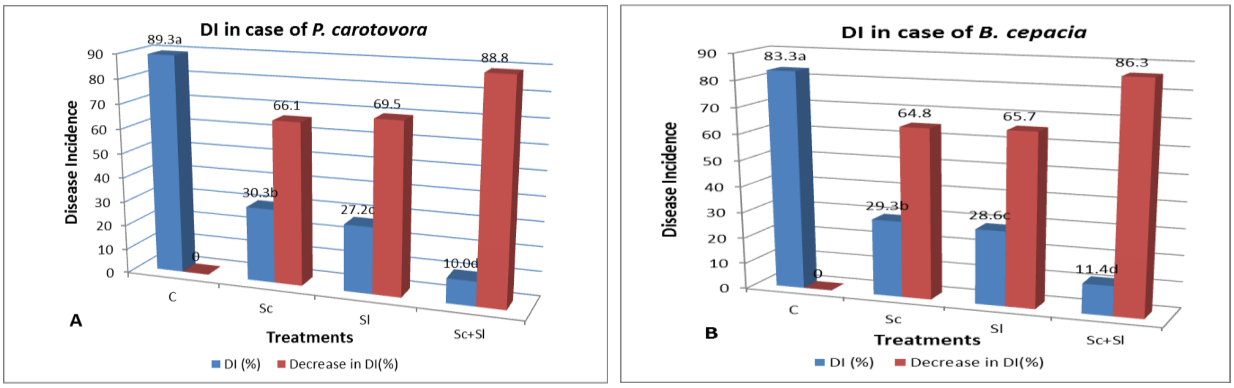


**Fig. 1. Effect of BCAs on the decrease percentage of onion bacterial rots incidence.**
**C = control, Sc = *S. coelicolor*, Sl= *S. lavendulae*.**

**Effect of BCAs on the antioxidant enzymes activities in onion infected with bacterial rot pathogens**

Data in Fig. 2 (A & B & C & D) clarified that application of *S. coelicolor and S. lavendulae* singly or in mixture have significantly increased activities of POX and PPO in treated onion. The highest increase was recorded for their mixed application where the increase percentage in POX activity was 80.4% and 87.3 % for *P. carotovorum* and *B. cepacia* treatments respectively, and the increase percentage in PPO activity was 37.1 % and 49.4 % for *P. carotovorum* and *B. cepacia* treatments respectively. The results are in agreement with Yousef *et al.* (2016); Badr and El-Sharkawy (2018) and El-Sharkawy *et al.* (2018 a,b) who reported that the application of *Streptomyces* increase the activity PPO and POX enzymes. POX enzyme stimulates the interaction of $Ca^{2+}$ signals required for inducing defence responses, phenols oxidation to quinine which are toxic to phytopathogens and taking part in cell wall polysaccharide processes like host plant cells lignification, suberization, and through the defence reaction against phytopathogens (Fayzalla *et al*., 2009; Hilal *et al*., 2016 and Shafie *et al*., 2016). PPO enzyme oxidizes phenols to more toxic quinones, likewise the cross linking of quinones with other phenols or protein forms a physical barrier to the pathogens entrance (El-Sharkawy, 2010; Shafie *et al*., 2016 and Farouk *et al*., 2017).

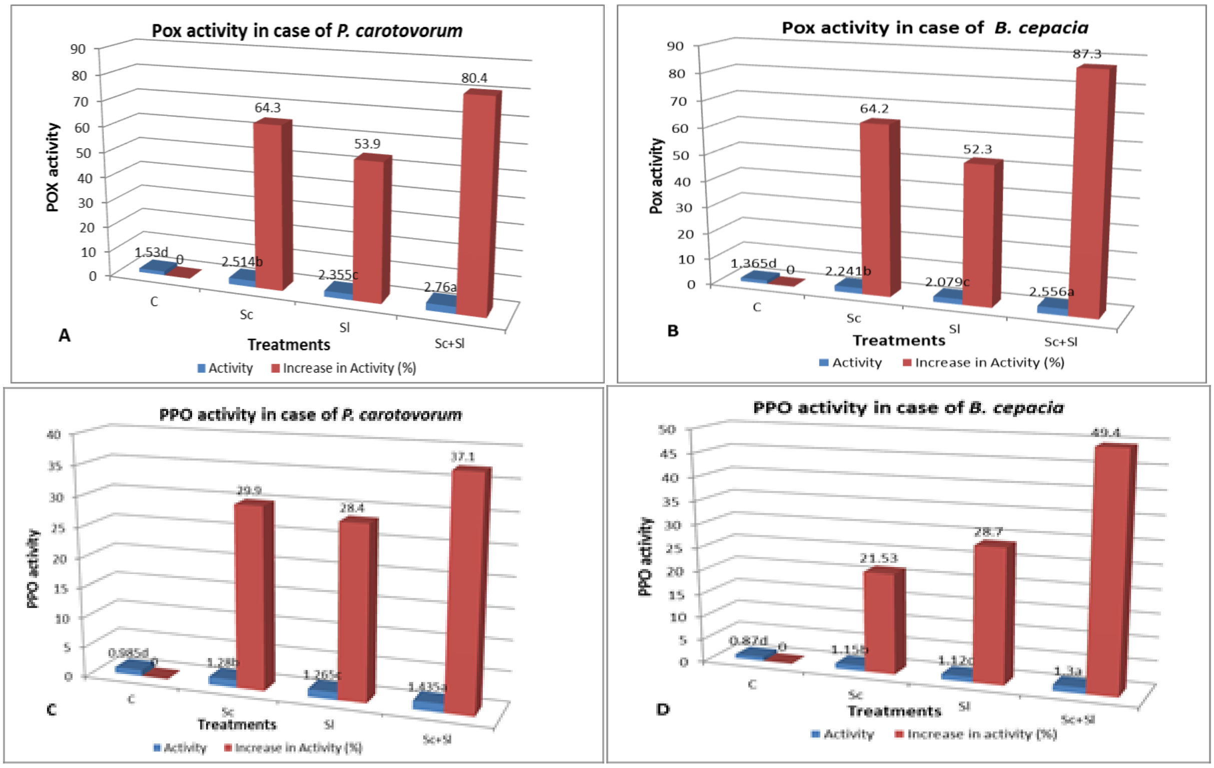


**Fig 2. Effect of BCAs on the antioxidant enzymes activities in onion infected with bacterial rot pathogens.**
**C = control, Sc = *S. coelicolor*, Sl= *S. lavendulae*.**

**Effect of BCAs on the total phenols content in onion infected with bacterial rot pathogens**

Data in Fig. 3 (A & B) showed that usage of *S. coelicolor* and *S. lavendulae* singly or in mixture significantly increased the total phenols in onion bulbs. The highest increase was recorded for their mixed application where the increase percentage was 356.1% and 353.8% for *P. carotovorum* and *B. cepacia* respectively. These obtained results are in harmony with those of Shafie *et al.* (2016); El-Sharkawy *et al.* (2018a) and Badr and El-Sharkawy (2018) who reported phenolics accumulation in plant cells due to *Streptomyces* application which also induce lignin biosynthesis in plant cell walls. Phenolics act as stimulators of plant defense genes, antimicrobial agents, growth inhibitors of invaders, and modulators of pathogenicity. They likewise act as precursors in lignin and suberin structure, participated in the forming of physical barriers for the attack (Farouk *et al.*, 2017; El-Sharkawy *et al.*, 2018a).

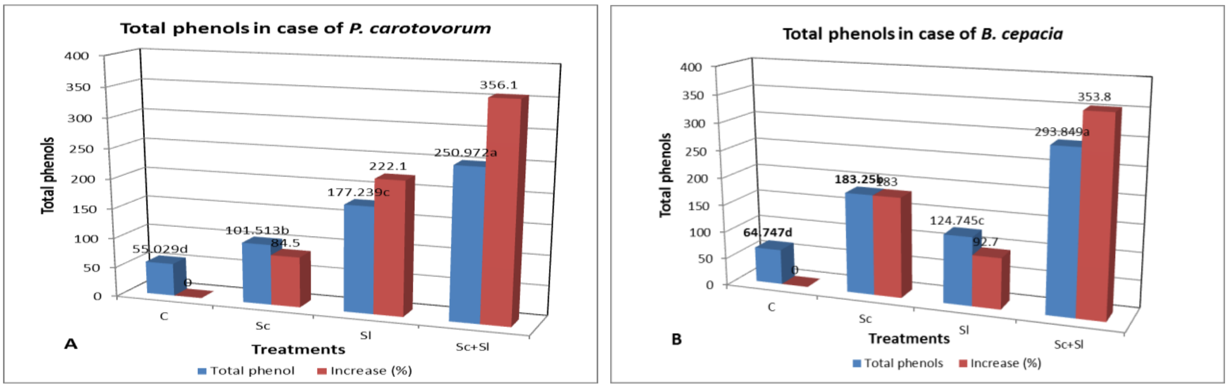


**Fig. 3. Effect of BCAs on the total phenols content in onion infected with bacterial rot pathogens.**
**C = control, Sc = *S. coelicolor*, Sl= *S. lavendulae*.**

**Effect of BCAs on Lipid Peroxidation in onion infected with bacterial rot pathogens:**

Results presented in Fig. 4 (A & B) indicated that there are significant decreases in the lipid peroxidation values in onion treated with *S. coelicolor* and *S. lavendulae* singly or in mixture. The highest decrease percentage was obtained by the mixed treatment as 53.3% and 57.7% in case of *P. carotovorum* and *B. cepacia*, respectively. The reactive oxygen species (ROS) accumulated in plant cells due to pathogen infestation which leads to an increase in the cellular lipid peroxidation which can be indicated by measuring the accumulated products of lipid peroxidation in plant cells as thiobarbituric acid reacting substances (TBARS) such as malondialdehyde. Application of BCAs decreased malondialdehyde (MDA) concentration in treated onion as compared withcontrol plants. The recorded results are in concord with that of Badr and El-Sharkawy (2018) who found that application of *S. coelicolor* and AMF decreased MDA concentration in onion bulbs.

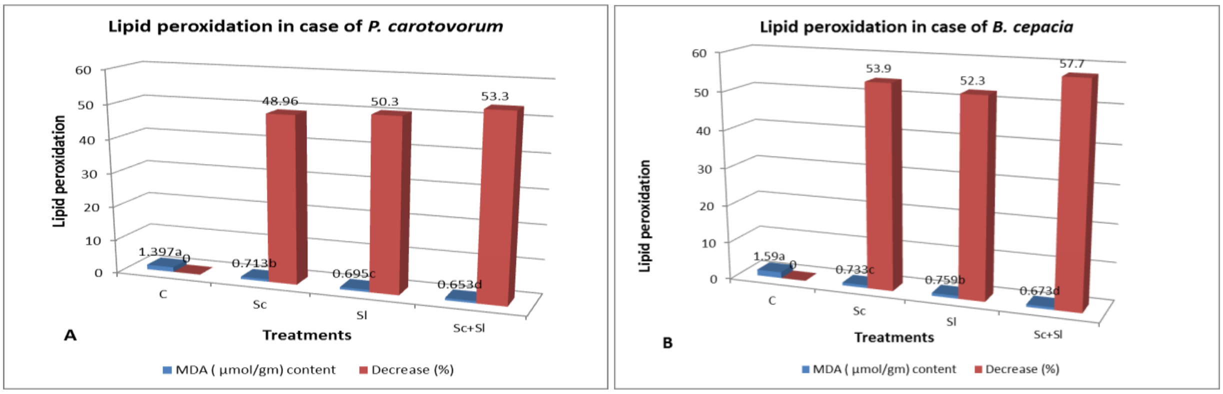


**Fig. 4. Effect of BCAs on lipid peroxidation in onion infected with bacterial rot pathogens**
**C = control, Sc = *S. coelicolor*, Sl= *S. lavendulae*.**

## CONCLUSIONS

Although *S. coelicolor* and *S. lavendulae* singly reduced onion bacterial rot disease incidence as well as increased the activity of PPO, POX and total phenols and decreased lipid peroxidation in onion bulbs, but their mixture was the best in this regard. Based on its eco-safety and efficiency, this mixture can be recommended to be used in controlling onion bacterial rots diseases.

**List of abbreviations:**

BCAs: biocontol agents; DI: disease incidence; C: control; Sc = *S. coelicolor*; Sl: *S. lavendulae*; PO: Peroxidase activity; PPO: Polyphenoloxidase activity.

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## تحسين كفاءة المبيد الحيوى من الأكتينوبكتيريا لمكافحة أمراض الأعفان البكتيرية فى البصل بخلط نوعين من الاستربتوميسس فى مصر

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**[١]قسم بحوث الأمراض البكتيرية- معهد بحوث أمراض النباتات-مركز البحوث الزراعية-الجيزه-مصر.**

**[٢]قسم بحوث الفطريات وحصر الأمراض- معهد بحوث أمراض النباتات-مركز البحوث الزراعية-الجيزه-مصر.**

أعفان البصل البكتيرية تعتبر من أخطر الأمراض التى تصيب البصل المخزن حيث تتسبب فى فقد كميات كبيرة من المحصول. العديد من الأنواع البكتيرية قد تسبب الأعفان للبصل ولكن أكثرها شيوعا *Pectobacterium carotovorum* ، *Burkholderia cepacia* وهما المستخدمان فى هذه الدراسة. تم إجراء هذه الدراسة كمحاولة لتحسين قدرة المبيد الحيوى من الأكتينوبكتيريا فى مكافحة أمراض الأعفان البكتيرية فى البصل وذلك بخلط *Streptomyces coelicolor* ، *Streptomyces lavendulae* فى معاملة واحدة واختبارها ضد كلا من *P. carotovorum* ، *B . cepacia* كلا على حده. قد تم تسجيل أعلى معدل لانخفاض نسبة حدوث المرض في معاملة الخلط بين *S. coelicolor* ، *S. lavendulae.* حيث سجلت ٨٨,٨% ، ٨٦,٣% فى حالة *P. carotovorum* ، *B . cepacia* على التوالى. وجد أن استخدام *S. coelicolor* ، *S. lavendulae* كلا على حدة أو خلطهما معا أدى إلى زيادة معنوية فى كمية الفينولات الكلية فى البصل ولكن أعلى زيادة تم تسجيلها فى المعاملة المختلطة لهما حيث كانت النسبة ٣٥٦,١% ، ٣٥٣,٨% فى حالة *P. carotovorum* ، *B . cepacia* على التوالى. كذلك وجد أن نشاط إنزيمات الأكسدة في البصل كانت أعلى باستخدام معاملة الخلط فى حالة *P. carotovorum* ، *B . cepacia*. على جانب آخر وجد أن نسبة اختزال الدهون في البصل المعامل قد انخفضت بشكل معنوى فى كل المعاملات ولكن أعلى نسبة اختزال قد سجلت فى حالة معاملة الخلط حيث كانت ٥٣,٣% ، ٥٧,٥% فى *حالة* *P. carotovorum* ، *B . cepacia* على التوالى. بناءا على الكفاءة والأمان البيئي لـ *S. coelicolor* ، *S. lavendulae* فقد يوصى باستخدامهما فى معاملة واحدة لتحسين كفاءة المبيد الحيوى من الأكتينوبكتريا لاستخدامه لمكافحة أمراض الأعفان البكتيرية فى البصل.