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Combined Effect of *Streptomyces coelicolor* and Arbuscular Mycorrhizal Fungi on Controlling Onion Bacterial Rots

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ABSTRACT

Bacterial rots are among the most serious diseases of onion that is being appeared in storage and/or transit. This research aimed to investigate effect of the combined application of *Streptomyces coelicolor*, and arbuscular mycorrhizal fungi (AMF) on controlling onion bacterial rots. *S. coelicolor* and AMF, individual or in combination, a significant decrease in the disease incidence was shown with superiority of the combined treatment. Total phenol content of plants and polyphenol oxidase and peroxidase enzymes were significantly increased in response to this application. However, a significant decrease in lipid peroxidation was recorded. According to their distinguished efficiency and known remarkable eco-safety, this combination can possibly be used in the biological control of bacterial rot diseases of onion. Further greater application may be tried.

Keywords: Onion, *Pectobacterium*, *Burkholderia,* Bacterial rots, AM fungi, *Streptomyces*, Biocontrol.

INTRODUCTION

Bacterial rots of onion are among the most serious storage diseases of bulbs that may be categorized into at least three different diseases; soft rot (caused by *Pectobacterium carotovorum* or *Dickeya chrysanthemi*), slippery skin (caused by *Burkholderia gladioli* subsp. *allicola*) and sour skin (caused by *Burkholderia cepacia*) (Chaput, 1995 McNab, 2004 and Mansour *et al*., 2011).

The rot bacteria are soil borne, but their symptoms on onion bulbs develop in storage and/or transit when the surrounding conditions are favored such as humid conditions and warm temperature. The bacterial rot symptoms include watery rot of the bulb fleshy scales and discoloration of the scales from pale yellow to brown, the rotten bulbs have an offensive smell (Mansour *et al*., 2011).

Onion bacterial rots were tentatively determined to cause yield losses ranging from 5 to 50 % (Schwartz and Gent, 2004), thus the need for effective safe control is of paramount importance.

Actinomycetes are good producers of a huge number of materials with biological activity that have high commercial values such as antibiotics, vitamins, plant growth stimulators, alkaloids, enzymes, enzyme inhibitors, ect. (Tanaka and Omura, 1993).

Arbuscular mycorrhizal fungi are obligate biotrophs which have a synergistic association with many plants. They play various beneficial function for the plant host, including bio-protection, bio-fertilization, bio-regulation, and alleviation of salinity and drought stresses (Fayzalla *et al.*, 2009 and Rouphael *et al*., 2015).

As *Streptomyces* and AMF are effective in controlling plant pathogens, they also help plants to mobilize and get nutrients (Perner *et al.*, 2006; El-Sharkawy, 2010 and Gopalakrishnan *et al.* 2014). They have different antagonistic mechanisms, including direct parasitism, antimicrobial compounds production and competition for nutrients and space (Aghighi *et al.,* 2004; Ara *et al.*, 2012 and Roatti *et al.*, 2013). Furthermore, AMF and *Streptomyces* may control the disease indirectly *via* compensation of root damage, morphological modification of the plant root, improvement of plant health, and/or induction the resistance of the plant (Abdel-Fattah *et al*, 2011, Majewska *et al*, 2017).

The present study aimed at 1) evaluating the individual and combined applications of *Streptomyces coelicolor* and AMF for controlling onion bacterial rot diseases under storage conditions and 2) estimation of some defensive biochemical parameters in the onion bulbs as a response to the treatment with the biocontrol agents.

MATERIALS AND METHODS

Bacterial inocula:

Two strains of the onion bacterial rot pathogens; *Pectobacterium carotovorum* and *Burkholderia cepaci* were used in this study, that were isolated and identified in a previous study by Badr (2011).

Bioagents:

Streptomyces coelicolor was isolated, identified and tested for *in vitro* antagonistic potential against *Pectobacterium carotovorum* and *Burkholderia cepacia* in a previous study by Badr (2011). A mixture of AM spores was used in this study. It composed of *Funneliformis mosseae* (Nicolson & Gerd.) Walker & Schüßler, *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) Walker & Schüßler, *Gigaspora margarita* (Becker & Hall), *Rhizoglomus clarum* (Nicolson & Schenck) Sieverd., Silva & Oehl and *G. gigantean* (Nicol. & Gerd.) Gerd. & Trappe (in equal proportions). The AM fungi were propagated for six months on sudan grass in pots filled with sterilized soil. AM fungi inocula were used as 50 g/pot according to El-Sharkawy (2010).

***In vivo* studies**

Pot experiment was carried out during 2015 in Dakahlia governorate, Egypt to evaluate the effect of merging *S. coelicolor* and AMF in one treatment for controlling onion bacterial rot diseases.

Onion transplants of red cultivar were planted in plastic pots with clay: sand soil (2:1), two transplants/pot), the pots were arranged into two groups; the first for studying the tested treatments against *Pectobacterium carotovorum* and the second for *Burkholderia cepaci.* The tested treatments; 1) *S. coelicolor*, 2) AMF, 3) *S. coelicolor* + AMF and 4) control, were added to the soil before onion transplanting. Each treatment in each group had 6 replicates. One month before harvest, the bacterial pathogens were inoculated in the soil each in its group as liquid inoculum with 106 CFU/ml.

After four months from transplanting, the yield was harvested and left 2 weeks in the open air to dry before storing.

Postharvest examinations

Determination of disease incidence:

After 10 months, the disease incidence was determined for the stored onion bulbs for each treatment according to the methods of Badr (2011) by the following formula

DI (%) = no. of diseased bulbs/ total no. of stored bulbs * 100

Biochemical analysis

Immediately after harvesting onion bulbs, the activity of the oxidative enzymes as polyphenol oxidase (PPO) and peroxidase (POX), total phenols content, and lipid peroxidation were determined.

Extraction and assay of polyphenol oxidase and peroxidase enzymes were performed according to the method described by Biles and Martyn (1993). Estimation of total phenol content was done according to the method described by Maliak and Singh (1980) using folin ciocalteau reagent.

The method of Shao *et al.* (2005) was used to estimate lipid peroxidation in the form of thiobarbituric acid reacting substances (TBARS), content of malondialdehye (MDA) was estimated and calculated as µmol/gram fresh weight.

Statistical analysis

Data were analyzed with the statistical analysis system CoStat (CoHort Software, U.S.A) version 6.4 (CoStat 2005). The means comparison was done using Duncan's multiple range test (Duncan 1995) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of *S. coelicolor* and AMF on the disease incidence of onion bulbs

Data in table (1) show that all tested biocontrol agents significantly decreased disease incidence of onion bacterial rots disease. The highest decrease in disease incidence of bacterial rots in case of *B. cepacia* and *P. carotovorum* was observed with the combined application of *S. coelicolor* and AMF that recorded 75.1 and 73.0% respectively, followed by *S. coelicolor* treatment (65.0, 65.2%) and then AMF treatment (61.6%, 60.3).

The results are in concord with those reported by Abdallah *et al*. (2013), who reported a significant protection by *S. coelicolor* against *B. cepacia* and *P. carotovorum* on onion bulbs, and are in harmony with results of Hayashida *et al*. (1988) and Mansour *et al*. (2008) on potato plants, the formers found that the use of a biofertiliser containing *Streptomyces albidoflavus* strain CH-33 that produces an antibiotic lethal to *Streptomyces scabies* gave the best decrease in DI of potato common scab, while the latter demonstrated that treatment of potato with *Streptomyces sioyaensis* which established *in vitro* antibacterial activity against *P. carotovorum*, the pathogen of potato soft rot, caused significant decrease in the soft rot DI throughout storage of potato tubers. AM fungi had ability to induce protection against different pathogen of plant diseases (Fayzalla *et al*. 2009 and El-Sharkawy 2010). Root colonization of onion by AMF triggers the resistance of plant and enhances several plant defense-related mechanisms. The jasmonic acid dependent signaling pathway regulates AM Fungi induced resistance (El-Sharkawy *et al* 2018b). In this regards, several physiological and physical mechanisms were discussed such as plant cell wall lignification, proteins and different antimicrobial compounds accumulation (such as phenolic compounds), induced the defense-related enzymes and enhanced hypersensitivity reactions (Abdel-Fattah *et al*. 2011). Also, some pathogenesis-related protein-encoding genes (chitinase and β-1,3-glucanase) induction was reported as a response to AM fungi colonization of bean plants against *R. solani* infection (Abdel-Fattah *et al*. 2011).

Application of biocontrol agents induced protection against plant diseases by several mechanisms as secretion of cellulase, chitinase and protease enzymes, these enzymes are induced during the parasitic interaction and can inhibit several plant pathogens growth by degrading its cell walls (Tanaka and Omura, 1993; Zhang *et al.* 2012; Saber *et al.* 2017), accumulation of the phenolic compounds is important in resistance of plant disease through delaying the invading pathogens growth, production of antibiotics those are toxic to phytopathogenic microbes (Aghighi *et al.,* 2004; Ara *et al*. 2012 and Mutitu *et al*. 2008). Furthermore these phenolics act as precursors in lignin and suberin synthesis, which act as physical barriers for plant invasion (Fayzalla *et al.* 2009; El-Sharkawy 2010; Surekha *et al.* 2013), and activation of antioxidant enzymes such as polyphenol oxidase and peroxidase, which involved in the oxidation process of phenolic compounds to more toxic quinones, accompanied with inactivation of pectinolytic enzymes produced by the pathogens, another suggested mode of action for PPO and POX is the increment of lignification and thickness of cell wall acting as a physical barrier for pathogen invasion (Doumbou *et al*., 2002; Surekha *et al*., 2013 and Al-Askr *et al.*, 2016).

Table 1. Effect of *S. coelicolor* and AMF on onion bacterial rot diseases incidence.

Treatment	*Burkholderia cepacia*		*Pectobacterium carotovorum*	
	D I (%)	Decrease %	D I (%)	Decrease %
Control	85.7[a]	00.0	90.0[a]	00.0
AMF	32.9[b]	61.6	35.7[b]	60.3
S. coelicolor	30.0[c]	65.0	31.3[c]	65.2
AMF+ *S. coelicolor*	21.3[d]	75.1	24.3[d]	73.0

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (*P* ≤ 0.05)

Effect of *S. coelicolor* and AMF on the activity of defence-related enzymes of onion infected with bacterial rot pathogen:

Plants resort to synthesis of diverse antioxidant enzymes to protect themselves against oxidative stress. It was observed that the activity of peroxidase and polyphenol oxidase was higher in bulbs treated by the biocontrol agents compared to the control. The highest activity was reached by the combined treatment of *S. coelicolor* and AMF (Table 2). In this regard El-Sharkawy (2010) reported a positive relationship between resistance in tomato and the peroxidase activity. POX is an oxidativereduction enzyme that stimulates the interacting Ca^{2+} signals required for oxidation of phenols, oxidation of phenols to toxic quinine to pathogen and take part in cell wall suberization, induction of defense responses, and lignification of host plant cells during the defense reaction against pathogenic agents (Nandakumar *et al.,* 2001; Fayzalla *et al*., 2009 and Hilal *et al.*, 2016).PPO which is a plastid copper enzyme was found to catalyse the oxygen-dependent oxidation of phenols to more toxic quinones, also the cross linking of quinones with other phenols or protein forms a physical barrier to the pathogens entrance (El-Sharkawy 2010; Al-Askr *et al.* 2016 and Shafie *et al.* 2016).

Effect of *S. coelicolor* and AMF on the total phenols content of onion infected with bacterial rot pathogen:

Data in Table (3) show that application of *S. coelicolor* and AM fungi singly or in combination significantly increased total phenols. The highest increase was recorded for their combined application where the increase percentage was 125.9 and 156.9% for *P. carotovorum and B. cepacia* treatments respectively. Secondary metabolites accumulation is another clue for the role of biocontrol agents in disease resistance. The obtained results demonstrated that biocontrol agents have evoked the phenolic compounds hyper accumulation in onion bulbs. Phenolics act as antimicrobial agents, growth inhibitors of invaders, activators of plant defense genes and modulators of pathogenicity. They also act as precursors in lignin and suberin synthesis, involved in the formation of physical barriers for the invasion (Surekha *et al.,* 2013 and Al-Askr *et al.,* 2016). Defense-related enzymes such as POX, PPO and TAL regulate the production of soluble phenolics. The results are in concord with those of El-Sharkawy (2010); Yousef *et al*., (2016) and El-Sharkawy *et al*., (2018a,b) who reported that the application of AM fungi and *Streptomyces* induced the accumulation of phenolics and biosynthesis of lignin in plant cell walls.

Table 2. Effect of *S. coelicolor* and AMF on the activity of defence-related enzymes of onion infected with bacterial rot pathogen

Treatment	POX activity (unit/g F.W.)				PPO activity (unit/g F.W.)			
	P. carotovorum		*B. cepacia*		*P. carotovorum*		*B. cepacia*	
	Activity	Increase %	Activity	Increase %	Activity	Increase %	Activity	Increase %
Control	0.510[c]	00.0	0.449d	00.0	0.197c	00.0	0.174d	00.0
AMF	0.762[b]	49.4	0.566b	26.1	0.280a	42.1	0.240c	37.9
S. coelicolor	0.725[b]	42.2	0.474c	5.6	0.256b	30.0	0.265b	47.1
AMF+*S. coelicolor*	0.838[a]	64.3	0.589a	31.2	0.282a	43.2	0.275a	58.1

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (*P* ≤ 0.05).

Table 3. Effect of *S. coelicolor* and AMF on the total phenols content of onion infected with bacterial rots

Treatment	Total phenols (mg/100 g fresh weight)			
	P. carotovorum		*B. cepacia*	
	Total phenol	Increase %	Total phenol	Increase %
Control	195.0c	00.0	164.47d	00.0
AMF	359.7b	84.5	337.528c	105.22
S.coelicolor	360.4b	84.8	349.549b	112.5
AMF+ *S. coelicolor*	440.5a	125.9	422.51a	156.9

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (*P* ≤ 0.05).

Effect of *S. coelicolor* and AMF on Lipid Peroxidation of onion infected with bacterial rots:

Data in the Table (4) showed that there are significant decreases in the value of lipid peroxidation in onion treated with *S. coelicolor* and AM fungi singly or in combination. The highest decrease percentage was recorded in the combined treatment as 50.2% and 54.9% in case of *P. carotovorum and B. cepacia,* respectively. One of the expected results of pathogen infestation-induced cellular build-up of reactive oxygen species (ROS) is an increase in lipid peroxidation. The assay of cellular accumulation of lipid peroxidation products, in the form of thiobarbituric acid reacting substances (TBARS), can supply a comparative indication of such activity. Application of biocontrol agent decreased malondialdehye (MDA) concentration in treated onion as compared with control plants.

Under pathogen infestation, the equilibrium between production and scavenging of free radicles oxygen is broken, resulting in oxidative damage of macromolecules. Lipid peroxidation in plants has been proven to be induced by pathogens, and the subsequent products have been appearing to pass antimicrobial properties and signaling function (Croft *et al*., 1993). In this regard Yang *et al*., 2015 found that application of AM fungi suppressed the increase in MDA concentration in plant tissue and membrane permeability.

Table 4. Effect of *S. coelicolor* and AMF on lipid peroxidation of onion infected with bacterial rot pathogen

Treatment	Lipid Peroxidation			
	P. carotovorum		*B. cepacia*	
	(MDA= µmol/gm)	decrease %	(MDA= µmol/gm)	decrease %
Control	1.429a	00.0	1.62[a]	00.0
AMF	0.745b	47.9	0.778[b]	52.0
S. coelicolor	0.736b	48.5	0.755[b]	53.4
S. coelicolor +AMF	0.712c	50.2	0.730[b]	54.9

Values in each column followed by the same letter are not significantly different according to Duncans multiple range test (*P* ≤ 0.05).

In conclusion, application of *S. coelicolor* and AMF was shown to be successful approach in the biological control of onion bacterial rots and the combination of them was superior. Regarding to their efficiency and eco-safety, it can be recommended to use this combination in controlling onion bacterial rots. Further pilot experiments may be tried on larger scale.

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التأثير المشترك لاستخدام بكتيرة استربتوميسيس كوليكلر و الفطريات الشجيرية الجذرية على مقاومة أمراض الأعفان البكتيرية في البصل

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تعد الأعفان البكتيرية من أخطر الأمراض التي تصيب البصل و تظهر أثناء التخزين و النقل و غالبا ما تسببها بكتيرة *Pectobacterium carotovorum* و *Burkholderia cepacia*يهدف البحث لاختبار التأثير المشترك لاستخدام بكتيرة استربتوميسيس كوليكلر و الفطريات الشجيرية الجذرية معا على مقاومة أمراض الأعفان البكتيرية في البصل فضلا عن استخدامهما كلا على حده. قد وجد أن استخدامهما منفردان أو مجتمعان أدى إلى انخفاض معنوي في نسبة حدوث المرض و لكن استخدامهما مجتمعان كان الأفضل. لوحظ زيادة نشاط انزيمي البروكسيديز و البولي فينول أوكسيديز و كذلك الفينولات الكلية في الأبصال نتيجة المعاملة بالاستربتوميسيس كوليكلر و الفطريات الشجيرية الجذرية و كانت الزيادة أكبر في المعاملة المشتركة لهما. كما و قد وجد انخفاض معنوي في نسبة أكسدة الدهون في البصل نتيجة المعاملة ببكتيرة استربتوميسيس كوليكلر و الفطريات الشجيرية الجذرية و كانت نسبة الانخفاض أكبر في المعاملة المشتركة لهما فضلا عن استخدامهما كلا على حده. بالإشارة إلى الفاعلية الواضحة للاستخدام المشترك لبكتيرة الاستربتوميسيس كوليكلر و الفطريات الشجيرية الجذرية في مقاومة أمراض الأعفان البكتيرية في البصل فضلا عن الأمان البيئي لهما، يمكن التوصية باستخدام هذه المعاملة المشتركة في المقاومة البيولوجية لأمراض الأعفان البكتيرية في البصل.