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post harvest control of bacterial soft rot pathogens of onion bulb in storage

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ABSTRACT

General characteristics of bacterial pathogens based on the biochemical and physiological tests indicated that 41 isolates Out of 88 bacterial isolates collected from rotted onion bulbs were identified as; *Erwinia cacticida* (2), *Erwinia carotovora* subsp. *atroseptica* (5), *Erwinia carotovora* subsp. *betavasculorum* (6), *Erwinia carotovora* subsp. *carotovora* (16), *Pantoea* spp (5) and *Burkholderia cepacia* (7). Storage trial for fresh harvest and cured onion bulbs collected from field plots irrigated, withholding irrigation four months before harvest and 4 different levels of fertilizers (N, K) treatments. Assessment of bacterial bulb rot disease incidence (DI%) were carried out every 2 months regular intervals during 10 months of storage for onion bulb samples after external treatments with propionic acid spray (0,025%), Streptomycin sulfate spray (100 ppm) and actinomycetes (*Streptomyces coelicolor*) dust formulation. Results indicated that highest DI % in check treatment after 10 months of onion bulb storage was 20.68%. Compared with this control treatment; significant reduction in DI% was found in most treatments after all periods of onion bulbs storage. The highly significant reduction in DI% attributed to spray with propionic acid were 2.13%, 1.51%, 1.27%, 0.67% and 0.0%; followed by dust treatment with actinomycetes were 4.32%, 3.71%, 3.23%, 1.86% and 1.50% after 2, 4, 6, 8 and 10 months; respectively. However; low significant difference or reduction in DI% was found between irrigation and withholding irrigation treatments. Among fertilizers treatments; N1K1 treatment (150kg N+ 24kg K2O/fed) showed significant reduction in DI% reached to 4.41%, 5.54%, 6.34% and 7.18% after 2, 4, 6 and 8 months of storage but after 10 months was not significant.

Keywords: Onion, soft rot bacteria, propionic acid, actinomycetes, storage, post-harvest control

INTRODUCTION

The annual cultivated area by onion in Egypt was115295 fed in 2010 season, this area produced 1563.3 thousand tons/fed with average of 13.56 tons/fed, as mentioned by the yearly book of Economics and Statistics of the Agriculture Ministry in Egypt. In onion production areas, bacterial contamination by the plant pathogen belonging to the soft rot species may be serious constraint to onion production and commercialization. These phytopathogens can cause bulb soft rot and can also result in the occurrence of various field symptoms, including reduced emergence, chlorosis, wilting, stem rot, blackleg, desiccation and plant death (Hélias *et al*., 2000). Bulb rotting can also develop both after harvest and from storage because soft rot bacteria are often carried as latent infection in symptomless onion bulbs(Hélias *et al*., 2000 and De Boer, 2002). This can cause further spread of infection and can lead to reduced yields and increased production cost. Bacterial soft rot is considered as one of the limiting factors of onion production in some areas of the world as well as in Egypt. High humidity and free water favour spread and penetration of the bacteria. Disease

development is dependent on high temperatures, generally 25-30°C.The effect of onion soft rot disease is more pronounced in the developing countries where appropriate storage facilities are lacking (Bdliya and Haruna, 2007). Onion production has been significantly affected due to soft rot disease caused by *Erwinia* spp. and *Burkholderia* spp; (Abdalla *et al*; 2013) in Egypt. However, no single method controlled the soft rot disease; but there are many agricultural practices that used to protect onion crop and reducing the disease such as balanced fertilizers, harvest time and curing and treatment the bulbs to prevent the disease to spread in storage. Development of suitable and environment friendly control measures of soft rot causing bacteria may minimize the loss in storage and improve the quality of onion. In Egypt; research work on the soft rot of onion are scanty and no effective controls measures have been developed for control of soft rot causing bacteria in storage. Considering the above facts; the present study aims to characterize and identify soft rot causing bacterial pathogens isolated from onion, to find out effective control measures against soft rotting bacterial pathogens in storage.

MATERIAL AND METHODS

1.1. Disease inspection and pathogen (s) isolation

Diseased onion bulb samples were selected based on visible symptoms showing water-soaking or yellowish brown color of soft rot and characteristic odor described by **Rich (1983)** and **Shing (2001)** Vertical cut was made in each bulb by knife to expose the internal scales to describe the rot symptoms. For bacterial pathogen (s) isolation; a small pieces of about 1 cm were removed from margins of the rotted onion bulb scales with a scalpel and suspended in 3 m sterile water, macerated and allowed to settle 15 min. One loop full of resulting suspension (water containing bacteria) streaked on the solidified dry plates of modified nutrient agar (mNA) medium (**Difco manual, 1953**). The plates were incubated at 28° C for 24 hr. Purification of bacterial colony was done by re-streaking of single colony on another fresh plate. A 10-fold-dilution series was prepared from suspensions of each sample extract and 100 mL of each dilution and the undiluted extract were spread on yeast extract-dextrose-calcium carbonate (YDC) medium (**Schaad, 2001**) and King's medium B with three replications of each dilution. Plates were incubated for 48 h at 30^0C. Colonies were re-streaked onto YDC plates for pure culture isolation. Bacterial isolates were purified and retained on YDC and KB slants at 48^0C for further tests of identification.

1.2. Pathogenicity (virulence) test

Healthy onion bulbs were used for the pathogenicity test. With the use of sterile toothpick, wounds were made on an onion bulb to inoculate the causal agent. The bacterial inocula were obtained from 2-day-old cultures on KB broth medium incubated at 30^0C. Bacterial cells were collected in saline phosphate buffer and adjusted to10^7 cfu/ml. The bacterial causal agent was inoculated longitudinally from the neck part and transversely from the outer to the inner part of the onion bulb at the level of 100 mL on wound. Inoculated

onion bulbs were incubated at 30^0C in a moist chamber for 7 days. Inoculated bulbs were examined daily for development of symptoms. The causal agent was re-isolated from onions bulbs showing the same symptoms of decaying and browning on King's Medium B.

1.3. Characterization and identification of bacterial isolates

All pathogenic bacteria isolates produced variable degrees of rot symptoms on onion bulbs were selected to subsequent identification tests. Some morphological, cultural and biochemical characters studies according to Schaad (2001) were done to differentiate presumptive pathogenic isolates to which bacterial genera belong. Characterization of the presumptive pathogen was carried out by subjecting the isolated bacterial colonies to various biochemical tests like Gram reaction, anaerobic growth, yellow colonies on YDC and NA media, fluorescent pigment on KB medium, growth on DIM and utilization of arginine. The common genera were subjected to further tests to identify their species according to Lelliott and stead (1987); Schaad (2001); De Boer and Kelman (2001); Chun and Jones (2001) and Sotokawa and Takikawa (2004).These tests include growth at 37^0C, reducing substances from sucrose, sensitivity to erythromycin, indole production, acid production, acid production from: lactose, inulin, cellobiose, glycerol and starch, oxidase, urease activity, and utilization of sucrose, maltose and D-tartrate. Soft rotting bacterial strains *Erwinia carotovora* subsp. *carotovora* and *Burkholderia cepacia* were used as reference strains in this experiment. Each test was conducted with three replicates for each strain and repeated twice.

1.4. Effect of antibacterial compounds on onion bulb rot in storage

Red onion cultivar (Giza 6) was used in the storage experiment. Onion bulb of new harvested crop that used in the storage represent 2 field treatment of irrigation (normal irrigation and withholding irrigation 4 weeks before harvest) and 4 different levels of Nitrogen (N) and Potassium (K) fertilizers as follow; N1K1 (150kg N+ 24kg K2O/fed), N1K2 (150kg N+ 12kg K2O/fed), N2K1 (120kg N+ 24kg K2O/fed) and N2K2 (120kg N+ 12kg K2O/fed). The first dose of fertilizer was applied after thirty days from planting and the second, one month later. Moreover; normal addition of Super phosphate (15.5% P2O5) and potassium sulphate (48% K2O) were applied during land preparation. The control treatment was bulbs received normal irrigation and fertilizer. Onion bulbs in all trials were harvested after about 4 months from planting (when 50% of the onion neck fallen down). Then onion was cured in the open air for 2 weeks before storage. Effective chemical and biological control treatments that tested against onion bulbs soft rot bacterial pathogens and inhibited its growth in laboratory were propionic acid (0.025 %), streptomycin (100 ppm) as spray solution and actinomycetes (*Streptomyces coelicolor*) as dust powder formulation. The three antibacterial treatments were applied to onion bulbs upon storage. Propionic acid (0.025%), streptomycin sulfate (100 ppm) solutions were sprayed using fine volume atomizer on all the surfaces of the stored bulbs as equal or uniform

thin wetted layer. Spray with sterile water only served as control or check treatment and fresh actinomycetes dust formulation with wheat bran was applied to onion bulbs while wheat bran dust only was served as check treatment. All the treated lots of each storage treatment were left to be air dried; then collected back to the storage location. Onion bulbs were stored in ambient storage in rows covered with rice straw for 10 months period as normal storage done by farmers in Egypt. By the end of every storage period; bulbs were screened for bacterial infection and disease incidence on regular intervals every 2 months after storage, percentages of bacterial rot disease in bulbs were estimated to the total number of the onion bulbs in each lot of all storage treatments.

Statistical analysis: All collected data were subjected to statistical analysis as described by **Snedecor and Cochran (1973)** Significance among means was tested using LSD method according to **Walter and Duncan (1969).**

RESULTS AND DISCUSSION

1.1. Characterization of the bacterial isolates

Out of 88 bacterial isolates isolated from infected onion bulbs from different onion storage locations; 41 isolates were Gram negative and selected on the base of its colony character and virulence tests compared with known onion soft rot reference strains. All isolates were categorized according to their similarity in their colony morphology on differential media of **Schaad (2001)** into three major groups as displayed in **Table (1)**.

1.2. Pathogenicity (virulence) test

All isolates were reisolated from artificially inoculated bulbs showing the symptoms were similar to those due to natural infections. The symptoms developed along the outer fleshy scales of onion after 7 days of artificially inoculation. Infected tissue is yellow initially, turning into brown as the disease progresses lengthwise in the bulb. Water-soaking (soft rot) developed gradually into inner part of onion bulbs.

1.3. Characterization and identification of bacterial isolates

All isolates were usually Gram negative; short rod-shaped, motile, and non-spore-forming. Based on the biochemical and physiological tests in **Table (2)** used to distinguish species and subspecies of the first group of 29 isolates designated as *Erwinia* spp (code No. I1-3, I2-3, I4-2, I11-1, I12-1, I17-2, I18-1, I18-2, I21-1, I22-2, I23-2, I24-1, I26-1, I27-1, I29-1, I1-2, I2-2, I3-1, I3-2, I3-4, I3-5, I4-1, I4-3, I7-2, I7-3, I8-4, I9-1, I13-1 and I20-1). On the basis of the following tests; all the 29 isolates and reference strain *Erwinia carotovora* produced yellow colonies on YDC agar plates and showed positive growth on MS medium. The isolates were found positive in catalase test, anaerobic growth but negative in oxidase test, do not produce fluorescent pigment on KB agar, sensitive to erythromycin and arginine utilization and they found variable O/F test, growth on 37^0C and reducing

substances from sucrose. These isolates were tested for utilization and acid production from D-Lactose, Inulin, Cellobiose, Glycerol, Starch and Trehalose on which two isolates (code no. I8-4 and I27-1) were found negative for D-Lactose and Inulin and positive for other substances and identified as ***Erwinia cacticida***. The rest of the isolates were positive for D-Lactose, Cellobiose, Glycerol and Trehalose and negative for starch utilization and variable for Inulin utilization. Based on the biochemical and physiological tests in five isolates were identified as *Erwinia carotovora* subsp. *atroseptica*, six isolates as *Erwinia carotovora* subsp. *betavasculorum* and sixteen isolates identified as *Erwinia carotovora* subsp. *carotovora*. On the other hand; 12 Gram negative isolates showed different biochemical and physiological results. The isolates do not produce yellow colonies on YDC agar plates and showed negative growth on MS medium. Those isolates were found positive in catalase test, sensitive to erythromycin. Out of 12 isolates 5 isolates were found negative for oxidase test and positive for anaerobic growth, growth on 37^{0}C, catalase test, reducing substances from sucrose and produce yellow colonies on YDC agar plates. The 5 isolates were identified as *Pantoea* spp. Also, Results indicated that all the 7 isolates and reference strain were positive for arginine utilization and negative results for growth on DIM agar plates, fluorescent pigment on KB agar plates and do not produce yellow colonies on YDC. All the isolates and reference strain were found positive for the growth on and utilization of Cellobiose, Trehalose, Sucrose and Maltose and negative for D-Tartrate. Based on the previous results, the 7 isolates were identified as *Burkholderia cepacia* (Table 2).

Regarding bacterial soft rot of onion bulbs our results were agreed with many research work on onion bulb soft that considered the disease as widespread as the soft rot is quiet destructive in storage condition. The results of physiological and biochemical tests and carbon sources utilization tests also revealed that major isolates of onion bulb rot were identical in basic tests with reference strain of *Erwinia carotovora* and *Burkholderia cepacia*. Similar type physiological and biochemical and carbon source utilization results for B. cepacia were reported earlier by (Kreig and Holt 1984; Schaad 1988; Khan 2000a).The soft rot of onion can be caused by *E. carotovora* subsp. *carotovora* (Shing 1985). This organism is a common cause of loss in stored onion (Sherf and Macnab, 1986). Several species of bacteria can cause soft rot depending on environmental condition and presence of the species (**Shing 1985**). Soft rots can spread in storage. It is also reported that acres affected 100% at risk and up to 75% can be affected (**Stivers 1997**).In the present study characterization and identification of soft bulb rot bacteria of onion were mostly based on the traditional methods. In our laboratory; molecular based techniques yet not been performed for characterization and identification of soft rot bacteria of onion so far. It may be due to lack of facilities. So molecular based techniques should be included in future for characterization and identification of soft rot bacterial strains.

1.4. Effect of antibacterial compounds on onion bulb rot in storage

Data presented in Table (3) displayed clear differences among stored onion bulbs samples represent all onion of different irrigation/fertilizers treatments that reflected on the storability and infection by postharvest diseases combined with effective chemical and biological control (propionic, streptomycin and actinomycetes) applied as post-harvest spray and dust formulations to control infection by soft rot bacteria. Results on assessment of bacterial bulb rot disease incidence (DI%) during 10 months of storage for onion bulb samples after external chemical or biological substances treatments presented in Table (3) showed that highest of DI % in check or control of onion bulbs without any treatment after 10 months of onion bulb storage was 20.68%. Compared with this control treatment; significant reduction in DI% was found in most treatments after all periods of onion bulbs storage. The highly significant reduction in DI% attributed to spray with propionic acid were 2.13%, 1.51%, 1.27%, 0.67% and 0.0%; followed by dust treatment with actinomycetes (4.32%,3.71%, 3.23%, 1.86% and 1.50%) after 2, 4, 6, 8 and 10 months; respectively. However; low significant difference or reduction in DI% was found between irrigation and withholding irrigation treatments. Among fertilizers treatments; N1K1 treatment (150kg N+ 24kg K2O/fed) showed significant reduction in DI% reached to 4.41%, 5.54%, 6.34% and 7.18% after 2, 4, 6 and 8 months of storage but after 10 months was not significant.

Onion is stored in ambient storage condition in our country where the storage losses are very high. It is estimated that 40- 50% of the stored onion never reaches to the consumers because of various types of losses. These losses are comprises of physiological loss in weight i.e. moisture losses and shrinkage (30-40 %), rotting (10-12 %) and sprouting (8-10 %).The higher storage losses were due to physiological loss of weight occurring during the months of May to July when mean temperatures are high. The rotting losses are high in the high humid months of rainy season. The sprouting of onion starts in September -October when the temperatures starts decreasing. In other research work; chemical sprays are generally not recommended for the control of soft rot (Agrios.1997b) although many scientists used various chemicals including bactericides and microbial pesticides to control the soft rot bacteria (Abd-EI-Khair 2007; Wright *et al*. 2005). Control of insects that spread the disease which can reduce the infections both in the field and in storage (Agrios, 1997b). Propionic acid was first registered as a pesticide in the early 1970's. In 1975, EPA first exempted propionic acid from certain tolerance, or legal residue limit, requirements. Currently, two manufacturers use pesticide registered products, each containing propionic acid as its sole active ingredient. Lam (2006) reported that Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolism. Abdalla *et al*., (2013) isolated 45 actinomycetes strains from Egyptian soils and screened for their antagonistic effect against onion bacterial rot pathogens; *Erwinia carotovora* subsp *carotovora* and *Burkholderia cepacia*. The most active strains were identified based on their cultural, morphological and molecular properties as *Streptomyces lavendulae*

and *Streptomyces coelicolor*; the latter was most potent and so was used for controlling onion bacterial rot. *S.coelicolor* application results in enhancement of photosynthetic pigments and some foliar growth parameters of onion plants confirming its growth promoting effect. The results of the post-harvest estimation of the disease incidence (DI) of the onion bacterial rot throughout storage revealed that application of *S. coelicolor* reduced markedly the DI compared with untreated control and confirm its successful role in the biological control of onion bacterial rot diseases. Streptomycin sulfate used in storage trial for onion soft rot control in the present investigation was not effective as propionic acid and actinomycetes under storage condition while it was effective under laboratory condition. This may be due to used concentration and the application method as external spray do not ensure efficacy in transmission of streptomycin to the inner scales of onion bulbs or it may be lost its efficacy and disintegrated as a chemical compound under high temperature during storage. Our data agreed with the findings of Tanaka and Aota (1990) examined the factors which affecting tissue decay inhibition with streptomycin to determine the reason for the failure in controlling bacterial soft rot with streptomycin. At 20 degrees C, streptomycin completely inhibited tissue decay caused by Erwinia carotovora subsp. carotovora. Decay, however, increased progressively with rise in temperature. In incubating slices for different periods at 30 degrees C and later at 20 degrees C, longer incubation at 30 degrees C also promoted tissue decay. Although repeated inoculation for as many as 5 times failed to promote decay, delayed treatment of streptomycin following inoculation resulted in extensive decay. Inoculation with a bacterial suspension incubated for more than 7 hours in the Japanese radish root caused extensive decay. The inoculation of bacterial cells collected from a bacterial suspension 24 hours after incubation also caused extensive decay. High temperature, delay in streptomycin treatment following inoculation, and inoculation. Ansary *et al.* (2006) studied the yield, quality and post-harvest life of onion under different moisture regimes (no irrigation, farmer's practice of 80- 10 days interval and irrigation at 0.55 and 0.80 atm. tension) and fertilizer rates (no fertilizers, N: K: S at 100: 120: 40 and 150: 180: 60 kg/ha). He found that highest neck thickness (1.59 cm), bulb diameter (4.58 cm), bulb weight (56.73 g), TSS (10.08 degrees Brix) and yield (225.72 q/ha) were obtained under irrigation 0.55 atm tension. Regarding the effect of irrigation and fertilizers on storability of onion storage our results are agreed with the findings of Wright and Grant (1997) found that the levels of bacterial soft rot in stored onion bulbs were affected by rates and application times of nitrogen (N) fertilizer, time of lifting, and water during the field-curing period. Onions received N late in the growing season had more storage rots than onions that received N early in the season, or not supplied with N. There were no noticeable differences in levels of rots in onions that were lifted at 50-70% top-down or at >90% top-down in each of the treatments. When N treatments were compared, the percentage of storage rots was higher in onions that were given supplementary water during field-curing than in non-irrigated onions.

Table (1): Characterization of bacterial isolates isolated from infected onion bulbs with different rot symptoms for morphology characters on agar plates and pathogenicity test.

Isolates characterization Categories			**Bacterial Isolates**
Group III	**Group II**	**Group I**	
Gram negative, on mNA medium plates its shape were smooth, round, glistening, slightly raised and some were flat to slightly raised, margins undulated to feathery and visible. Also; it had yellow color to pale beige to white, creamy white, and grayish creamy white color,	Gram negative, on mNA medium plates had circular, convex with entire margin and glistening shape; grown on YDC agar plates as non-yellow colonies, no fluorescent pigment on King's B agar plates.	Gram negative, on YDC agar medium without yellow color and positive growth on MS agar plates. All colonies had the whitish to creamy or yellowish color and colony shapes of smooth, raised to convex or circular shape.	**Colony character**
(5)	(7)	(29)	**(Total No of isolates**
Positive	Positive	Positive	**Pathogenicity test** for onion soft rot symptoms
n.d.***	**-**	**+**	**Ref. strain I ***
n.d.	**+**	**-**	**Ref. strain II****

***, Ref. I = Reference strain of Erwinia carotovora subsp. carotovora.**
****, Ref. II =Reference strains of Burkholderia cepacia.**
*****, Reference strain not available (n.d.= not done)**

Table (2): General physiological and biochemical teststo differentiate soft rot bacterial Genera, species and subspecies of Erwinias, Pantoea and Borkholdrias

Characteristics	*Results*		
	Erwinias	*Pantoea*	*Burkhold-erias*
- **Gram staining**	G–ve rods	G–ve rods	G–ve rods
- **Anaerobic growth**	+	+	-
- **Yellow Colonies on YDC**	-	+	-
- **Growth on MS agar medium**	+	-	**n.d***
- **Growth on DIM agar medium**	**n.d**	-	+
- **Growth on 37^{o}**	+	+	-
- **Gram reaction (KOH)**	+	+	+
- **Catalasetest**	+	+	+
- **Oxidasetest**	-	-	+
- **Reducingsubstancesfrom sucrose**	-	+	+
- **Sensitivityto Erythromycin**	-	+	+
- **Utilization of Arginine**	-	-	+
- **Fluorescent pigment onKing's B medium**	-	-	-
Utilization of carbon source (acid production)			
- **D-Lactose**	+	-	-
- **Inulin**	+	-	-
- **Cellobiose**	+	-	+
- **Glycerol**	+	-	-
- **Starch**	-	-	-
- **Trehalose**	+	-	+
- **Maltose**	**n.d.**	-	+
- **D- tartarate**	**n.d.**	-	+

* n.d. = not done

Table (3): Assessment of bacterial bulb rot disease incidence (DI%) during 10 months of storage for onion bulb samples drawn from individual field experiment of irrigation and fertilization treatments and samples treated with external chemical or biological substances.

Treatments	DISEASE INCIDENCE (%)				
	Storage intervals (Month)				
	2 M	4 M	6 M	8 M	10 M
Irrigation treatments					
Irrigation	5.309	6.416	7.411	8.436	10.054
withholding	5.058	6.266	6.925	8.089	9.672
F. test	*	NS	NS	*	NS
Fertilization treatments					
N1K1 *x	4.406 c	5.540 c	6.340 a	7.183 c	9.186 b
N1K2	5.085 b	6.205 b	7.170 a	8.414 b	9.792 b
N2K1	5.153 b	6.308 b	7.102 a	8.033 b	9.418 b
N2K2	6.089 a	7.311 a	8.059 a	9.420 a	11.058 a
F.test	**	**	*	**	**
Spray /dust treatments					
Propionic acid	2.126 d	1.509 d	1.269 b	0.668 d	0.000 d
Streptomycin	6.562 b	8.685 b	10.781a	13.143 b	17.268 b
Actinomycets	4.315 c	3.708 c	3.226 b	1.857 c	1.502 c
Control	7.731 a	11.462 a	13.395 a	17.382 a	20.683 a
F. test	**	**	**	**	**

[x] N1K1 = (150kg N+ 24kg K2O/fed), N1K2 = (150kg N+ 12kg K2O/fed), N2K1 = (120kg N+ 24kg K2O/fed), N2K2 = (120kg N+ 12kg K2O/fed)

**= significant, ** = highly significant, NS= not significant - Values followed by the same letter(s) in each column do not differ significantly (*$P \leq 0.05$*)*

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مقاومة المسببات المرضية بعد الحصاد للعفن الطرى البكتيرى فى البصل اثناء التخزين

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تم تجميع عينات بصل مصابة بمرض العفن الطرى البكتيرى والحصول منها على ٨٨ عزلة بكتيرية وتم تعريفهم على اساس الاختبارات الفسيولوجية والبيوكيمائية فوجد ان من بينهم ٤١ عزلة بكتيرية ممرضة وتم تعريفهم كالاتى: *Erwinia cacticida* (2), *Erwinia carotovora* subsp. *atroseptica* (5), *Erwinia carotovora* subsp. *betavasculorum* (6), *Erwinia carotovora* subsp. *carotovora* (16), *Pantoea* spp (5) and *Burkholderia cepacia* (7). وتم اجراء تجربة تخزين للابصال الناتجة من تجربة حقلية طبق فيها ٢ معاملة للرى و٤ مستويات مختلفة من التسميد النتروجينى والبوتاسى(N, K) حيث استخدمت الابصال المسمطة جيدا وتم معاملتها ب٣ معاملات مضادة لنمو البكتريا قبل التخزين وهى الرش بحمض البروبيونيك بتركيز(٠,٠٢٥%) والرش بالمضاد الحيوى استربتوميسين بتركيز(١٠٠جزء فى المليون) والتعفير بمستحضر من الاكتينوميستات من جنس (*Streptomyces coelicolor*) ثم التخزين لمدة ١٠ شهور والفرز كل شهرين مع حساب شدة الاصابة بالمرض لكل معاملة. ومن اهم النتائج المتحصل عليها ان اعلى نسبة اصابة بالمرض بعد ١٠ شهور من التخزين كانت٢٠,٦٨% مقارنة بالكنترول. ولوحظ انخفاض معنوى فى نسبة الاصابة فى معظم المعاملات بعد كل فترات التخزين. وقد تبين ان اعلى انخفاض معنوى فى نسبة الاصابة كانت فى معاملة الرش بحمض البروبيونيك حيث كانت نسب الاصابة على مدار شهور التخزين كالاتى: ٢,١٣ %، ١,٥١%، ١,٢٧%، ٠,٦٧%، ٠,٠% ثم يأتى فى المرتبة الثانية المعاملة بالتعفير لمستحضر الاكتينوميستات وكانت نسب الاصابة كالاتى:٤,٣٢%، ٣,٧١%، ٣,٢٣%، ١,٨٦%،١,٥٠%، وكذلك يوجد ايضا فروق معنوية او انخفاض معنوى فى نسبة الاصابة فى معاملات الرى والتسميد. ومن بين معاملات التسميد وجد ان المعاملة (N1K1 (150kg N+ 24kg K2O/fed اظهرت انخفاض معنوى فى نسبة الاصابة وصلت الى ٤,٤١%، ٦,٣٤%، ٥,٥٤% و ٧,١٨ % بعد٢، ٤، ٦ و٨ شهور من التخزين ولكن بعد ١٠ شهور من التخزين لم يكن هناك اى تأثير معنوى للمعاملة.

قام بتحكيم البحث

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