

Plant Protection and Pathology Research

http://www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master



EFFECTIVE FACTORS ON ONION BACTERIAL SOFT ROT DISEASE INCIDENCE DURING STORAGE

Asmaa A.M. Akar*, Entsar E.A. Abbas, M.R.A. Tohamy and H.M. El-Said

Plant Pathol. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 15/05/2019; Accepted: 10/06/2019

ABSTRACT: Seven bacterial isolates were isolated and identified from green onion and mature bulb (*Allium cepa* L.) that showing naturally infected soft rot disease. Onion samples used for isolation were collected from different super markets and store houses of Abou-Hamad District, Sharkia Governorate, during 2016/2017 seasons. Seven isolates showed different degree of aggressive pathogenic capability on onion bulbs. Morphological, physiological and biochemical diagnostic properties of the seven bacterial isolates confirmed that one isolate identified as *Bacillus polymyxa*, three isolates as *Pseudomonas cepacia* and three isolates as *Pectobacterium carotovorum* subsp. *carotovorum*. The seven identified bacterial isolates were compared for their degree of pathogenic capabilities in some different plant hosts. With the exception of chili fruits, the seven bacterial isolates were able to infect fruits, tubers, pods and roots of seventeen host plant species related to seven plant genera. Percentage of rotted onion bulb during storage significantly affected by the packing materials that used in preparing storage package, onion cultivar, maturity of bulb, type of cultivated soil and storage time. Results revealed that good control against onion soft rot could be obtained when mature bulbs of resistant cultivar cultivated in clay soil conducted to good cure program and stored under low temperature and high ventilation conditions.

Key words: Onion soft rot, *Bacillus polymyxa*, *Pseudomonas cepacia*, *Pectobacterium carotovorum*, packing materials, storage time.

INTRODUCTION

Onion (Allium cepa L.) is one of the important economic plants and widely cultivated in the world (Awuah et al., 2009). It is a biennial vegetable grown as an annual world wide. The uses of onion date back in food nutrition more over 4,000 years beyond the beginnings of written history (Necola, 2007). Onions are used in salads, cooked, eaten raw and for soups. Besides making a significant nutritional contribution to the human diet, onions also have medicinal and functional properties (Lanzotti, 2006).

Soft rot is considered the most destructive disease of vegetables. It occurs worldwide wherever fleshy storage tissues of vegetables and ornamentals are found. The disease can be

* Corresponding author: Tel.: +201284092303 E-mail address: sossoakar@yahoo.com found on crops in the field, in transit and in storage or during marketing resulting in great economic losses. The soft rot of onion is caused by E. carotovora subsp. carotovora (Shing, **1985**). This organism is a common cause of loss in storage (Sherf and Macnab, 1986). Postharvest diseases caused by bacterial pathogens include the species of soft rotting genera Erwinia, Pseudomonas, Xanthomonas, Cytophaga and Bacillus (Liao and Wells, 1987). A momental number of diseases have been reported in onion and cause production loss in the field and during storage were reported by Khan (1987). Pseudomonas Meah and marginalis, Ps. syringae and Ps. cepacia caused onion bulb rot in the field and in market places (Choi and Han, 1990). Vegetables in fields may already be infected even though they do not show visible symptoms at harvest. This latent

infection might cause severe post-harvest damage because of high temperature and humidity. Pectobacterium carotovorum subsp. carotovorum (formerly Erwinia carotovora subsp. carotovora) has been found to be the most common bacterial pathogen associated with soft rot diseases (Larka, 2004). This bacterium enters plant tissues primarily through wounds, often created by insect feeding or bruising at harvest or during post-harvest handling. The conditions for the development of the disease become favorable with increase in temperature during the summer. The disease proliferates rapidly and within no time, results in rapid tissue breakdown and thus causes heavy damage especially in countries lacking cold storage and with poor transportation and handling system (Higashio and Yamada, 2004). Post-harvest bacterial soft rot causes greater total loss of products than any other bacterial disease and have been estimated to vary between 15-30% of the harvested crop (Agrios, 2006). This study aimed to isolate and identify the bacterial pathogens causing soft rot of onion bulb by biochemical and physiological standard methods. Also to evaluate different factors including cultivars, soil type, maturity stage, package material and storage time on percentage of onion soft rot incidence during storage.

MATERIALS AND METHODS

Isolation and Purification of the Causal Organism

Forty bacterial isolates were isolated from naturally infected onion bulb (Allium cepa L.) that showing identical symptoms of onion soft rot. All samples were collected from different super market and store houses of Abou-Hamad District, Sharkia Government, Egypt during 2016/2017 season at different times. Collected samples were kept in paper dry pages and transferred to the pathological lab, Plant Pathology Dept., Faculty of Agriculture, Zagazig University. The collected diseased onion samples (green and bulb) were washed with tab water several times and sterilized in 2% sodium hypochlorite solution for 3 minutes, rinsed twice in sterilized distilled water. Small portion of the diseased tissues adjacent to the

healthy one were macerated with 5 ml of sterilized distilled water in sterilized pestle and mortar to exude bacterial ooze suspension. After 10 minutes, a loopful of the resulting suspension was streaked onto Nutrient Agar (NA) medium using streak plate technique described by Mortensen (1997) and Kim et al. (2002). Plates were incubated at 28°C for 24 hr., and then examined for bacterial growth development. The single colony technique was used to obtain pure culture. Single colonies of isolates were subcultured onto NA medium in tubes and maintained at 4°C for further studies (Schaad et al., 2001).

Preservation of Pathogenic Bacterial Strains

Pure single colonies of each of pathogenic soft rot bacterial isolates of onion were preserved in test tubes containing sterilized tap water. For long term storage, the bacteria were kept in Luria Bertani (LB) broth medium (Maniatis et al., 1982), supplemented with 20% glycerol and kept at -80 °C and were revived by plating on nutrient agar medium.

Pathogenicity Test

Healthy onion bulbs cultivars Giza 6 (red cv.) and Giza 20 (yellow cv.) were kindly obtained from Field Crops Research Institute, ARC. And used for the pathogenicity test. Wounds were made in the middle and near the neck of the onion bulb using cork bores (0.5 cm in diameter) and inoculated with 2 days old cultures (10⁸cfu/ml) of the bacterial isolates grown on KB broth medium (King et al., 1954) individually by sterilized toothpick. Each isolate was used to inoculate three replicates from onion bulbs. Control treatments were identically with sterile distilled water only. tested Inoculated onion bulbs were incubated at 28°C for 7 days under aerobic and anaerobic (in polyethylene pages) condition. Bulbs were examined for development of symptoms. The obtained results were calculated and statistically analyzed according to Gomez and Gomez (1984) by using MSTAT statistical program was used for analysis of variance (ANOVA). The causal agent was re-isolated from onion bulbs showing the same symptoms of decaying and browning on King's B medium to confirm Koch's postulates.

Characterization and Identification of Bacterial Isolates

The seven selected pathogenic bacteria isolates produced variable degrees of soft rot symptoms on onion bulbs, which were selected to subsequent identification tests. Some morphological, physiological and biochemical characters were studied according to Schaad et al. (2001) to differentiate between pathogenic isolates as well as to which bacterial genera belong. Characterization of the presumptive pathogen was carried out by subjecting the isolated bacterial colonies to various biochemical and physiological tests including Gram reaction, anaerobic growth, spore forming, motility, hydrolysis of casein, fat decomposition, gelatin liquefaction, starch hydrolysis, potato soft rot and fluorescent pigment on KB medium. King's B medium was used for detection of fluorescent green or blue pigment by inoculating tubes or plates with tested bacterial isolates and incubated at 28-30°C for 24-48 hr. After incubation period the growth was observed under UV light (King et al., 1954). Further tests were made to identify their species according to Kiraly et al. (1970), Lelliott and Stead (1987), De Boer and Kelman (2000), Schaad et al. (2001), Sotokawa and Takikawa (2004) and Rahman et al. (2017). These tests included growth at 41°C, reducing substances from sucrose, sensitivity to erythromycin, indole production, acid production from: D- arabinose, cellobiose, ethanol, fructose, lactose, mannose, trehalose, αmethyl- gluconases and sucrose. oxidase and urease activity. Each test was conducted with three replicates for each strain and repeated twice. The obtained results from these chemical and physiological properties were confirmed according to Don et al. (2005).

Host Range (Aggressiveness Degree)

The seven pathogenic bacterial isolates were inoculated into 17 plant species related to eight genera are listed in Table 1.

Aggressiveness degree was performed on different vegetables (Table 1) by inoculating them with the tested isolates individually in the middle of the plant organ (fruits, pods, tubers, roots and bulbs). The plant organ surfaces were

disinfected with 70% ethyl alcohol, rinsed with sterile distilled water and air dried. The plant organs were then wounded by sterilized cork borer (0.5 cm in diameter and 1 cm in depth) and inoculated by sterilized hypodermic syringe with 0.1 mm bacterial suspension (10⁸ cfu/ml) grown on nutrient broth (NB) medium for 48 hr., old cultures with three replicates of all hosts. then wounds closed by vaspar 1:1 (paravine wax+ vasline V:V). Samples of control were inoculated with sterilized distilled water. All species were then separately packed into sterilized polyethylene bags with sterilized wet filter paper to maintain humidity inside the bag. Bags were kept at room temperature for 7 days until symptom development. All the rotted lesions were measured by volume of rot and data were recorded for determination the most aggressive isolate (Ismail et al., 2012).

Effect of Cultivars, Maturity Stages, Soil Types and Package Materials on the Percentage of Onion Soft Rot Development during Twenty Weeks Storage under Ambient Room Temperature

Onion bulbs Giza 6 (red) and Giza 20 (yellow) cultivars were cultivated and grown in clay and sandy soil and harvested at two stages of physiological maturity. The first portion of the onions in the trial were harvested when 50% of the plants foliage had collapsed (top-down) on (15 April 2018). The second portion was harvested when they had maturity 90% (topdown) on 15 may 2018. Onion bulbs, on both occasions, were left on the ground to dry (curing) for 15 days. After field curing, the onion bulbs were transported to the packing shed. The dried tops were then mechanically removed and the bulbs were packaged in different package materials i.e. {Drifter made from Gunny (DG), Flour Drifter made from Plastic (FDP), Plastic Net Sack (PNS) and Bags made from Polyethylene (BP)} which were stored at ambient room temperature (25-30°C) and relative humidity (40-45% RH) for 20 weeks. Each replicate contained onion bulb (10 kg). Samples of onion bulbs were examined fortnightly against fungal free bacterial soft rot associated with strong smell of acetic acid and sometimes with gas bubbles (Bhat et al., 2012). Data were calculated as percentage of rotted onion bulb and statistical analysis was carried out according to Gomez and Gomez (1984).

Table 1. List of plant species tested for their reaction to the seven bacterial isolates

Host (common name)	Scientific name	Family name	Organ
Carrot	Daucus carota	Umbelliferae	Roots
Chili	Capsicum frutescens	Solanaceae	Fruits
Cucumber	Cucumis sativus	Cucurbitaceae	Fruits
Eggplant	Solanum melongena var. esculenta	Solanaceae	Fruits
Faba bean	Vicia faba	Leguminosae	Pods
Garden bean	Phaseolus vulgaris	Leguminosae	Pods
Garden pea	Pisum sativum	Leguminosae	Pods
Garlic	Allium sativum	Alliaceae	Bulb
Lemon	Citrus lemon	Rutaceae	Fruits
Pepper	Capsicum annuum	Solanaceae	Fruits
Potato	Solanum tuberosum	Solanaceae	Tubers
Pumpkin	Cucumis meloflexuosus	Cucurbitaceae	Fruits
Radish	Rphanus sativus	Cruciferae	Roots
Squash	Cucurbita pepo	Cucurbitaceae	Fruits
Sweet potato	Ipomoea batatas	Convolvulaceae	Roots
Tomato	Lycopersicon esculentum	Solanaceae	Fruits
Turnip	Brassica rape	Cruciferae	Roots

RESULTS AND DISCUSSION

Isolation and Identification

Among the forty examined isolates, previously isolated from rotted onion bulbs collected from different supermarkets and store houses at Sharkia Governorate, seven isolates proved to be pathogenically active against onion bulbs. Onion soft rot was clearly expressed four days after inoculation. Six isolates were Gram-negative, one isolate was Gram-positive (isolate No. 1) according to the obtained results in Table 2. The seven isolates were motile with flagella, nonsporular (except isolate No. 1), grown at 41°C and 5% Nacl, facultative anaerobic, hydrolysis casein and pectine. oxidase positive, decomposition fat and capable to produce soft rot on potato slices and negative erythromycin sensitivity. None of the isolated bacteria capable to produce indole. Most isolates

were capable to liquefy gelatin and grow on 7% Nacl in the exception of isolate No.1. Different results were obtained between tested isolates when examined for catalase production, pigment production on (KB) medium. All isolates were capable to produce acid or acid and gas from tested carbohydrate sources as shown in Table 2. These results are in harmony with the results obtained by Rahman et al. (2017). According to the results of morphological and physiological properties of the tested pathogenic bacterial isolates recorded in Table 2 and the results of properties reported by Obi and Umezurike (1981), Wright and Hale (1992) and Don et al. (2005) the isolates were categorized into three groups and were identified as Bacillus polymyxa, Ps. Cepacia and Pectobacterium carotovorum subsp. carotovorum (formerly Erwinia carotovora subsp. carotovora. Bacillus polymyxa has pectolytic activity and common in soil and frequently associated

Table 2. Some morphological, physiological and biochemical characteristics of the seven onion bacterial soft rot pathogenic isolates

Character							
	Bacillus polymyxa (1)	Pseudomonas cepacia (A) (2)	Pseudomonas cepacia (B) (3)	Pseudomonas cepacia (C) (4)	Pectobacterium carotovorum subsp carotovorum (A) (5)	Pectobacterium carotovorum subsp. carotovorum (B)	Pectobacterium carotovorum subsp. carotovorum (C) (7)
Growth at 41°c	+	+	-	-	+	+	+
Gelatine liquification	_	+	+	+	+	+	+
Starch analysis	+	-	_	-	-	-	-
Facultative anaerobic	+	+	+	+	+	+	+
Gram stain	+	-	-	-	-	-	-
Catalase	-	+	-	-	+	+	+
Casein hydrolysis	NT	+	+	+	+	+	+
Oxidase	NT	+	+	+	+	+	+
Pectinase	+	+	+	+	+	+	+
Growth at 5% Nacl	+	+	+	+	+	+	+
Growth at 7% Nacl	-	+	+	+	+	+	+
Morphology shape	Ū	d Short rod	Short rod	Short rod	Short rod	Short rod	Short rod
Motility	+	+	+	+	+	+	+
Fat decomposition	+	+	+	+	+	+	+
Sporulation	-	-	-	-	-	-	-
Pigment production on KB medium	-	-	+	+	-	-	-
potato soft rot	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-
Sensitivity to erythromycin	-	-	-	-	-	-	-
Fermentation of carbohydrate							
D- arabinose	A	A	A	A	AG	AG	AG
Cellobiose	A	A	A	A	AG	AG	AG
Ethanol	A	A	A	A	Α	A	A
Fructose	Α	A	A	A	A	A	A
Lactose	A	A	A	A	AG	A	AG
Mannose	Α	A	A	A	AG	A	AG
Trehalose	Α	A	A	A	A	A	A
α-Methyl-gluconases	-	-	-	-	A	A	A
Sucrose	-	-	-	-	A	A	A

A: production acid from carbohydrate G: production gas from carbohydrate NT: Note tested.

plants. It has been shown to cause rot of various plant tissues usually at high temperature and/or slight lack of oxygen. It may be particularly important in storage, but its effect can usually be avoided by good storage condition. It causes rots after harvesting and associated with *Erwinia carotovora* subsp. *carotovora*. This result was also in agreement with those reported by **Rahman** *et al.* (2017) who reported that the bacteria was found and isolated from rotted stored onion bulbs. Isolates No. 2, 3 and 4

related to be *Pseudomonas cepacia* according to the obtained results confirmed to the identification results obtained by **Choi and Han** (1990). Identification results of isolates No. 5, 6 and 7 showed that they were related to be *Pectobacterium carotovorum* subsp. *carotovorum* according parallelism to the taxonomy study of **Dye** (1969). The nature of previously organism is strongly pectolytic, rapid- growing and readily invades plant tissues if any entry is gained. It is therefore able to attack a large number of

different plants when conditions are suitable. The majority of host plants including onion have been recorded as natural hosts. Larger fleshy organs are particularly susceptible and once infected, they usually become softened to a pulp very quickly.

Pathogenicity Test

The seven isolates were re-isolated from artificially infected onion bulbs showing resemble symptoms and were comparable to the bulb natural infection. Results obtained from pathogenicity test in Table 3 reveal that isolate No. 7 (Pectobacterium carotovorum C) was the most aggressive one causing soft rot in onion tissues reached to 28.88cm3 in infected red onion bulb (cv. Giza 6) under anaerobic condition. In general the amount of soft rotted tissues were lower under aerobic condition than in anaerobic. These results are in harmony with those obtained by Wright and Hale (1992), Huang et al. (2003) and Raju et al. (2008). The soft rotted tissues of yellow onion bulb (cv. Giza 20) were significantly lower compared to red onion cultivar when inoculated by all tested bacteria. Different in cultivar susceptibility might by due to their genetic make-up (Agrios, **2006**). It could be arrange the isolates according to their ability to cause soft rot under anaerobic in red onion cultivar in descending order as P. carotovorum C, P. carotovorum A, P. carotovorum B, Ps.cepacia B, Ps.cepacia C, B. polymyxa and Ps. cepacia A and rot volume amounted as (28.88 cm³), (20.25 cm³), (19.30

cm³), (18.25 cm³), (14.70 cm³), (13.39 cm³) and (11.32 cm³), respectively. Infected yellow onion cultivar under anaerobic atmosphere exhibited lower amount of soft rot incidence and rot volume was aranged in descending order as *P. carotovorum* C, *P. carotovorum* A, *Ps. cepacia* C, *Ps. cepacia* B, *P. carotovorum* B, *B.polymyxa* and *Ps.cepacia* A and rot volume amounted as 18.98cm³, 12.24cm³, 11.51 cm³, 10.87cm³, 10.84 cm³, 09.14 cm³ and 06.89 cm³, respectively. Worthwhile, from aforementioned results and their statistical analysis, it could be concluded that, the interaction between isolates, variety and aeration were highly significant.

Host Range

As shown in Table 1 and Fig. 1 *P. carotovorum* C, A and B isolates were the most virulence that causing the highest rotted tissue volume in all tested hosts. On the other hand, B. polymyxa, Ps. cepacia A, B and C isolates were less effective causing the lower rotted tissues in tested hosts. Volume of rotted tissues was differed according to the virulence of isolate and the host susceptibility. Results in this point are similar with other studies (Mahmud and Monjil, 2015; Rahman et al., 2017). Turnip plant exhibited the most soft rot volume being (40.83 cm³) followed in descending order by carrot (38.80 cm³), pumpkin (23.28 cm³), green faba bean (21.19 cm³), eggplant (20.81 cm³), squash (20.43 cm³), cucumber (19.44 cm³), papper (16.64 cm³), tomato (16.47 cm³), sweet potato (13.71 cm³),

Table 3. In vivo pathogenicity test of onion bacterial soft rot disease incidence

No. of	Isolates	Red onion	bulb (Giza 6)	Yellow onion bulb (Giza 20)		
isolate		Aeration	An aeration	Aeration	An aeration	
1	Bacillus polymyxa	07.26*	13.39	04.85	09.14	
2	Ps. cepacia A	06.70	11.32	03.82	06.89	
3	Ps. cepacia B	08.53	18.25	04.71	10.87	
4	Ps .cepacia C	08.45	14.70	05.79	11.51	
5	P. carotovorum A	13.55	20.25	08.17	12.24	
6	P. carotovorum B	12.42	19.30	07.71	10.84	
7	P. carotovorum C	18.60	28.88	12.03	18.98	

^{*}Amount of soft rotted tissues (cm³).

LSD at 5% for: Isolates= 1.78 Variety = 0.95 Aeration = 0.95

Interaction:

LSD at 5% for:

Variety x Aeration = 1.35 Variety × Isolates = 2.52

potato (13.14 cm³), radish (12.07 cm³), pea (7.72 cm³), lemon (6.85 cm³), bean (4.83 cm³) and garlic (4.22 cm³), respectively. On the other hand, no soft rot developed on chili fruits was observed. Large number of vegetable species belongs to different families are known to infecte by Pectobacterium carotovorum, the causal organism of bacterial soft rot were reported by Bhat et al. (2010) and Ayesha et al. (2013). Bradbury (1986) has represented the wide host rang of E. carotovora. Also, Rajeh and Khlaif (2000) isolated eighty seven isolates of E. carotovora from soft rot of different vegetable crops. It is worthy to mention that the amount of rotted tissue values obtained from each host tested is considered as the result of interaction between the aggressiveness degree of isolate as well as the shape, size and degree of host susceptibility.

Effect of Cultivars, Maturity Stages, Soil Types and Package Materials on the Onion Percentage of Soft Rot **Development** during **Twenty** Weeks **Ambient Storage** under Room **Temperature**

Results related to the effect of different storage factors including onion cvs. Giza 6 (red) and Giza 20 (yellow), at two harvest maturity growth stages (50% and 90%) and moisture content for Giza 6 and Giza 20 onion bulbs were 88.2% and 87.2%, respectively are illustrated in Table 4 and Fig. (2 a,b,c,d). These cultivars were grown in clay and sandy soils. The harvested bulbs were packaged in four different sacks {Drifter made from Gunny (DG), Flour Drifter made from Plastic (FDP), Plastic Net Sack (PNS) and Bags from Polyethylene (BP)}. Onion bulb packages were stored for 20 weeks.

Results obtained revealed that, rotted onion bulbs packaged in different package materials, generally increased with increasing storage time. This effect was more pronounced and reached 100% soft rot before end of storage time when immature bulbs (50 % growth maturity stage) of the two tested cultivars were stored in all types of package materials used compared with the matured bulbs with less moisture content (90% growth maturity stage). Bulbs of Giza 6 (red cv.) was found to be more susceptible for rot than Giza 20 (yellow cv.). Increasing rots in immature

growth bulbs might be due to high moisture content compared with less rot associated with less moisture content in matured bulbs. This might be due to that quantifying soil water storage is important for plant growth and agricultural production are largely affected by the amount of water in the soil. This is particularly important in sandy soils because they are widely cultivated and more irrigate due to prone to draught (Jenifer and Alfred, 2019). Also, plastic polyethylene bags (BP) increased RH around stored bulbs and so, increased moisture content of bulbs which encouraged bacterial growth and infection resulted in increasing rots. These results are in agreement with those obtained by Mahmud and Monjil (2015). Mature onion bulbs (90% growth stage) grown in clay soil exhibited less soft rot compared with those grown in sandy soil. Results also showed that less bacterial soft rot calculated in both yellow and red onion cv. in onion bulbs harvested at 90% maturity growth stage than 50%. Similar results were obtained by Wright and Grant (1997).

In all cases, onion bulbs grown in clay soil and stored after harvest at different periods reached to 20 weeks, were more resistant against bacterial soft rot. This is due to less moisture content arround onion bulb grown in clay soil. Percentage of rotted onion bulb affected by the materials that used in preparing the storage sacks was differed according to the material used. Plastic net sack was more suitable package used to store both of red and yellow onion bulbs compared with other used package materials. On the other hand, sacks made from polyethylene encouraged the heights bacterial soft rot of onion bulb. This is due to increase RH around the bulbs associated with less ventilation that reduced the activity of oxidative enzymes specially polyphenol oxidase. This enzyme responsible for changing phenolic compounds into quinons that toxic to the bacteria (Araji et al., 2014). Sacks from gunny and plastic were between in their effect to use in stored onion bulb. In this regard, packing material was reported to be influence against bacterial soft rot and manifest the potential for controlling Pectobacterium carotovorum subsp. carotovorum soft rot as reported by Wright et al. (1993) and Bhat et al. (2012).



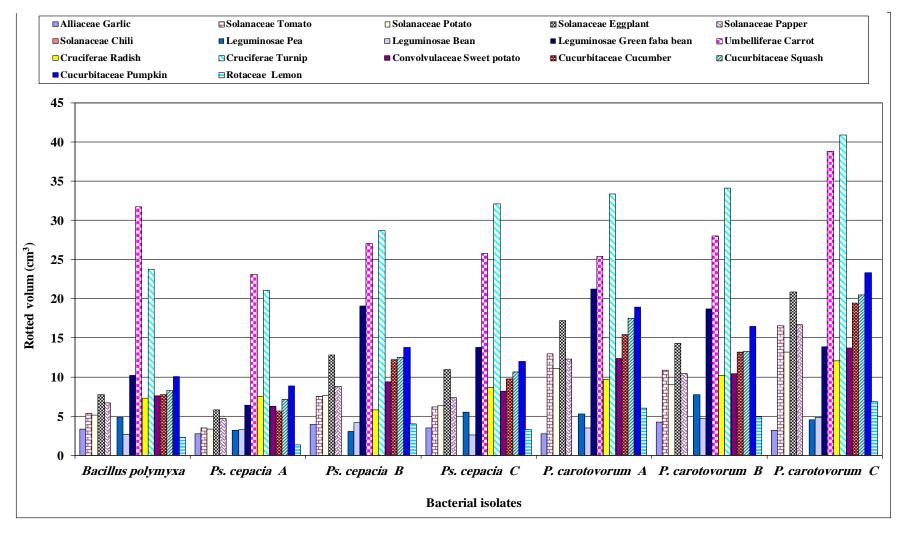


Fig. 1. In vivo effect of artificial inoculation by seven bacterial isolates causing onion soft rot measured as tissue rotted volume of seventeen host plants

Table 4. Statistical analysis of different package materials, maturity, onion cultivar, soil type, storage period and percentages incidence of soft rot during storage

Main effect and interaction	Infection (%)
Onion cultivar	
Giza 6 (red)	40.35 A
Giza 20 (yellow)	29.68 B
LSD Soil	0.513
S1 (clay)	31.27 B
S2 (sandy)	38.76 A
LSD	0.513
Maturity	
M1 (50%)	56.86 A
M2 (90%)	13.16 B
LSD Poskage	0.513
Package P1 (DG)	30.76 C
P2 (FDP)	38.57 B
P3 (PNS)	26.28 D
P4 (BP)	44.44 A
LSD	0.736
Period/two week	10.24 *
P1	10.24 J
P2 P3	12.46 I 15.85 H
P4	21.37 G
P5	29.09 F
P6	38.32 E
P7	46.09 D
P8	54.41 C
P9	59.84 B
P10 LSD	62.45 A 1.148
Interaction	1.146
Variety × Soil	
LSD	0.726
Variety × Maturity	
LSD	0.726
Variety × Package	1.027
LSD Variety v Pariod	1.027
Variety × Period LSD	1.624
Soil × Maturity	1.024
LSD	0.726
Soil × Package	
LSD	1.027
Soil × Period	1.624
LSD Maturity y Pagkage	1.624
Maturity × Package LSD	1.027
Maturity × Period	1.027
LSD	1.624
Package X Period	
LSD	2.297

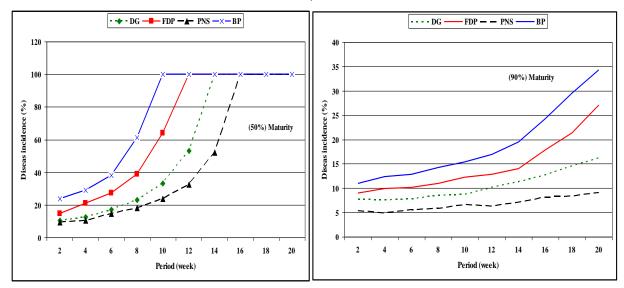


Fig. 2-a. Effect of different storage package materials at 50% and 90% maturity of red onion cultivar grown in clay soil type on percentage of soft rot incidence during storage period

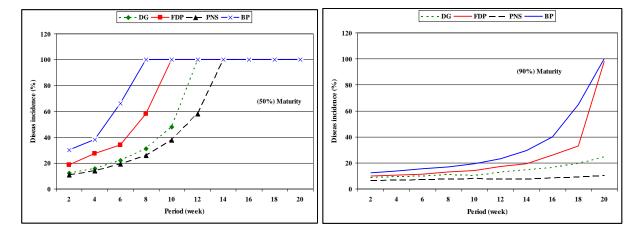


Fig. 2-b. Effect of different storage package materials at 50% and 90% maturity of red onion cultivar grown in sandy soil type on percentage of soft rot incidence during storage period

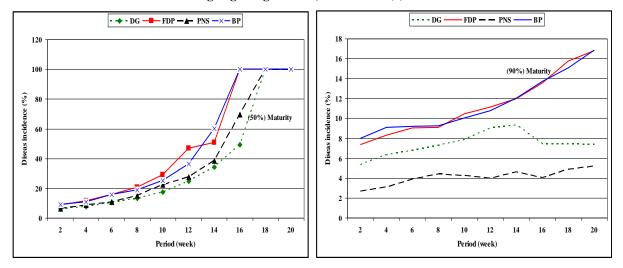


Fig. 2-c. Effect of different storage package materials at 50% and 90% maturity of yellow onion cultivar grown in clay soil type on percentage of soft rot incidence during storage period

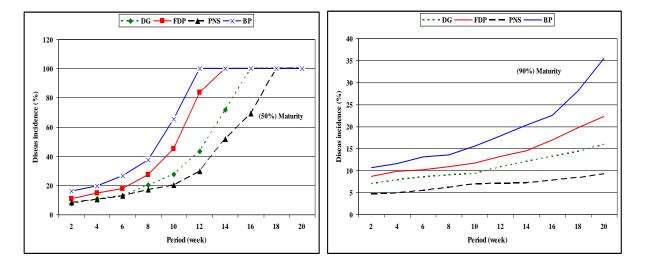


Fig. 2-d. Effect of different storage package materials at 50% and 90% maturity of yellow onion grown in sandy soil type on percentage of soft rot incidence during storage period

DG: Drifter from Gunny FDP: Flour Drifter from Plastic
PNS: Plastic Net Sack BP: Bags from Polyethylene

Least significant difference analysis in Table 4 revealed that, there is a significant difference between factors and their interaction used in this storage experiment. In this respect cultivar Giza 6 (red cv.) significantly was more susceptible due to higher moisture content than lowest moisture content in Giza 20 (yellow cv.) during storage time. Stored onion bulbs grown in clay soil significantly exhibited less rot compared with those grown in sandy soil. Also, bulbs harvested from mature onion plants (90% growth maturity stage) were significantly resistant during storage time than those harvested from immature onion plants (50% growth maturity stage). Significant differences were detected between the tested package materials used. PNS material was the more suitable as stored package followed by DG then FDP. On the other hand, BP was found to be less suitable package material for storing onion bulbs. Increasing storage time leads to significant increased rots.

Conclusion

Our conclusion illustrated that good control against onion soft rot could be obtained when mature bulbs of resistant cultivar cultivated in clay soil conducted to good cure program and stored under low temperature and high ventilation conditions.

REFERENCES

- Agrios, G. N. (2006). Bacterial Soft Rots. 5th Ed., Academic Press, San Diego.
- Araji, S., F.A. Grammer, R. Gertzen, S.D. Anderson, M. Petkovsek, R. Veberic, M.L. Phu, A. Solar, C.A. Leslie, A.M. Dandekar and M.A. Escobar (2014). Novel roles for the polyphenol oxidase enzyme in secondary metabolism and the regulation of cell death in walnut. Plant Physiol., 164 (3):1191-1203.
- Awuah, R.T., C.K. Woseh, S.L. Kuranteng, R.O.C. Okpala and I. Amoako-Attah (2009). Appearance of Fusarium Basal Rot Disease of Onion in the Kwahu Sought District of Ghana. Ghana J. Hort., 7:84-88.
- Ayesha, B., R. Abdur, J. Muhammad and A. Musharaf (2013). Aggressiveness studies of the pathogen of the bacterial black leg/soft rot of potato. Sarhad J. Agric., 29 (2): 219-224.

- Bhat, K.A., N.A. Bhat, F.A. Mohiddin, S.A. Mir and M.R. Mir (2012). Management of post-harvest Pectobacterium soft rot of cabbage (*Brassica oleracea* var *capitata* L.) by biocides and packing material. Afr. J. Agri. Res., 7 (28): 4066-4074.
- Bhat, K.A., S.D. Masood, N.A. Bhat, M.A. Shraf-Bhat, S.M. Razvi, M.R. Mir, S. Akhtar, N. Wani and M. Habib (2010). Current status of post harvest soft rot in vegetables: A review. Asian J. Plant Sci., 9 (4): 200-208.
- Bradbury, J.F. (1986). Guide to Plant Pathogenic Bacteria. CAB Int. Mycol. Inst., Ferry Lane, Kew, Surrey, England.
- Choi, J.E. and K.S. Han (1990). Studies on the bacterial soft rot disease of lilliaceae crops. 3- Rot of garlic caused by *Pseudomonas* spp. Korean J. Plant Pathol., 6: 86-90.
- De Boer, S.H. and A. Kelman (2000). Gramnegative bacteria: Erwinia soft rot group. In Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Ed. Ed., W., 56-72.
- Don, J.B., N.R. Krieg and J.T. Staley (2005). Bergy's Manual of Systemic Bacteriology. Second Edition, Volume Two. The Proteobacteria, Prt B. Introductory Essays. Williams and Wilkins, New yourk.
- Dye, D.W. (1969). A taxonomy study of the genus Erwinia. II. The "carotovora" groub. N.Z.J. Sci., 12: 81-97.
- Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agricultural Res., John Wiley and Sons, New York, (2nd Ed.).
- Higashio, H. and M. Yamada (2004). Control of soft rot after harvest of cabbage in Indonesia. Japan Agric. Res. Q., 38 (3): 175-178.
- Huang, H.C., T.F. Hsieh and R.S. Erickson (2003). Biology and Epidemiology of *Erwinia rhapontici* causal agent of pink seed and crown rot of plants. Plant Pathol. Bulletin, 12: 69-76.
- Ismail, M.E., M.F. Abdel-Monaim and Y.M. Mostafa (2012). Identification and pathogenicity of phytopathogenic bacteria associated with soft rot disease of girasole tuber in Egypt. J. Bacteriol. Res., 4 (1): 1-8.
- Jenifer, L.Y. and E.H. Alfred (2019). Effect of carbon on moisture storage in soils of the

- Wisconsin Central Sands, USA. Eur. J. Soil Sci., 1-13.
- Kim, Y.K., S.D. Lee, C.S. Choi, S.B. Lee and S.Y. Lee (2002). Soft rot of onion bulbs caused by *Pseudomonas marginalis* under low temperature storage, The Korean Soc. Plant Pathol., Plant Pathol. J., 18 (4): 199-203.
- King, E.O., M.K. Ward and D.E. Raney (1954). Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med., 44: 301-307.
- Kiraly, Z., Z. Klement, F. Solymosy and J. Voros (1970). Methods in Plant Pathology. Akademi aikiado, Budapest, 142-152.
- Lanzotti, V. (2006). The analysis of onion and garlic. J. Chromatography A, 1112 (1-2): 3–22.
- Larka, B.S. (2004). Integrated approach for the management of soft rot (*Pectobacterium carotovorum* sub-sp. *carotovorum*) of radish (*Raphanus sativus*) seed crop. Haryana J. Agron., 20:128-129.
- Lelliott, R.A. and D.E. Stead (1987). Methods for the diagnosis of bacterial diseases of plants. In: Methods in Plant Pathology; (2). British Soc. for Plant Pathology. Oxford, Blackwell Scientific Publications.
- Liao, C.B. and J. M. Wells (1987). Diversity of pectolytic, fluorescence pseudomonas causing soft rot of fresh vegetables at produce market. Phytopathology, 77: 673-677.
- Mahmud, M.S. and M. S. Monjil (2015). Storage diseases of onion under variable conditions. Progressive Agric., 26: 45-50.
- Maniatis, T., E. F. Fritsch and J. Sambrook (1982). Molecular Cloning: a Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Meah, B. and A.A. Khan (1987). Check list of vegetables and fruit disease in Bangladesh. Dept. Plant Pathol., Bangladesh Agric. Univ., Mymensingh, 22.
- Mortensen, C.N. (1997). Seed Bacteriology laboratory guide, Danish Govt. Ins. Seed Pathol. (DGISP) for developing countries, Copenhagen, Denmark, 1-2.
- Necola, S. (2007). *Allium cepa* variety evaluation for organic farming in the 5th int. symposium

- on Edible Allianceae. Dronten the Nether lands. ISHS. 45.
- Obi, S.K.C. and G.M. Umezurike (1981). Pectic enzyme activities of bacteria associated with rotted onions (*Allium cepa*). Applied and Environmental Microbiology. Univ. Nigeria, Nsukka., 42 (4): 585-589.
- Rahman, M.M., M.A.A. Khan, I.H. Mian, A.M. Akanda and M.Z. Alam (2017). Characterization of onion soft rot bacteria in Bangladesh. Bangladesh J. Sci. Ind. Res., 52 (3): 209-220.
- Rajeh, O.N. and H. Khlaif (2000). Soft rot disease of vegetables in Jordan, host range, reaction of some potato cultivars to the infection and effect of planting date. Dirasat Agric. Sci., 27: 149-157.
- Raju, M.R.B., V. Pal and I. Jalali (2008). Inoculation method of *Pectobacterium carotovorum* subsp. *carotovorum* and factors influencing development of bacterial soft rot in Radish. J. Mycol. Plant Pathol., 38: 311-315.
- Schaad, N.W., J.B. Jones and W. Chun (2001). Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Ed. APS Press, St. Paul. 373.
- Sherf, A.F. and A.A. Macnab (1986). Vegetable diseases and their control, 2nd Ed., A Wiley Interscience Publication, John Wiley and Sons, Inc. New York.
- Shing, R.S. (1985). Diseases of Vegetable Crops, Oxford and IBA Publishing Company Pvt. Ltd., New Delhi, 331.
- Sotokawa, N. and Y. Takikawa (2004). Occurrence of bacterial rot of onion bulbs caused by *Burkholderia cepacia* in Japan. J. Gen Plant Pathol., 70: 348-352.
- Wright, P.J. and C.N. Hale (1992). A field and storage rot of onion caused by *Pseudomonas marginalis*. New Zealand J. Crop and Hort. Sci., (20): 435-438.
- Wright, P.J., R.G. Clark and C.N. Hale (1993). A storage soft rot of New Zealand onions caused by *Pseudomonas gladioli* pv. *alliicola*. New Zealand J. Crop and Hort. Sci., 21: 225-227.
- Wright, P.J. and D.G. Grant (1997). Effect of cultural practices at harvest on onion bulb quality and incidence of rots in storage. New Zealand J. Crop and Hort. Sci., 25: 353-358.

العوامل المؤثرة على ظهور مرض العفن الطري البكتيري في البصل أثناء التخزين

أسماء عبدالغفار محمد عكر - إنتصار السيد عبدالنبي عباس محمد رضا أحمد تهامي - هاني محمد السعيد

قسم أمراض النبات – كلية الزراعة – جامعة الزقازيق – مصر

تم تعريف سبع عز لات بكتيرية عزلت من البصل الاخضر والأبصال الجافة المصابة طبيعيا بمرض العفن الطري، جمعت هذه العينات من السوبر ماركت وبيوت التخزين بمركز أبو حماد بمحافظة الشرقية مصر خلال موسم جمعت هذه العينات من السوبر ماركت وبيوت التخزين بمركز أبو حماد بمحافظة الشرقية مصر خلال موسم فيما بينها في درجة شراستها، أظهر التعريف بإستخدام الصفات المورفولوجية والفسيولوجية والكيميائية للعز لات السبع أن إحداها يتبع بكتريا Pseudomonas cepacia وثلاثة عرزلات تتبع Pseudomonas cepacia وثلاثة تتبع عندريا Pectobacterium carotovorum subsp. carotovorum على إصابة الأعضاء النباتية (ثمار درنات جذور قرون) لسبعة عشر نوع نباتي تتبع سبع أجناس نباتية، أوضحت على إصابة الأعضاء النباتية (ثمار درنات جذور عرون) لسبعة عشر نوع نباتي تتبع سبع أجناس نباتية، أوضحت الدراسة أيضا التأثير المعنوي لصنف البصل والتربة المزروع بها ودرجة نضج الأبصال المخزنة والمواد المستخدمة في تصنيع عبوات التعبئة وفترة التخزين على كمية العفن الناتج بعد التخزين لمدة ۲۰ أسبوعا. ولقد ازداد العفن البكتيري في أبصال الصنف الأحمر المزروع في التربة الرملية والتي تم حصادها مبكرا قبل تمام نضجها، وخزنت في أكياس من البلاستيك مقارنة بالصنف الأصفر المزروع في تربة طينية والمخزن في أكياس مثقبة.

۱ ـ د. نجــلاء بـــلابـل ۲ ـ أ.د. أحمد زكي علي