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Investigation on the Effect of Chemical and Biological Control of Bacterial Soft Root Disease of Potato in Storage.

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Abstract

Potato soft rot, caused by Erwiniacarotovora subsp. carotovora, greatly affect potato tuber quality in storage of Egypt and indicated that it can affect all potato cultivars. Erwiniacarotovora subsp. carotovora was isolated from the infected potato tuber and was identified by pathological, morphological and biochemical studies. One antimicrobialchemical compound chitosans (CS) with concentration (1, 3, 5 %) combine with three biocontrol agents (Bacillus subtilis, Pseudomonas fluorescens and Trichodermavirdi) each. These treatments were screened out in vitro and in storage against the growth of Erwinia carotovora subsp carotovora. All bio-control agents combined with Cs reduced the bacterial soft rot disease to various degrees. The stronger antagonistic activity against Erwinia carotovora was found in treatment Cs 5% with biocontrolagaints (T. virdi, P. fluorescens and B. subtilis respectively). All treatments reduced the soft rot infection to 20-week storage with two types of potatoes (Spunta and Cara) varieties by (91, 86, 83.6 and 77.3% respectively in Spunta c.v.) and (88.6, 86.4 and 79.8% in Cara c.v.). The lowest antagonistic activity against Erwinia carotovora was found in treatment CS 1% with biocontrol agents (T. virdi, P. fluorescens and B. subtilis respectively) varieties by (64.2, 58.6 and 43.7% respectively) compared with the treatments of biocontrol agents individually.

Keywords: Potato - Erwiniacarotovora- Chitosan- T. virdi - P. fluorescens- Bacillus subtilis

1. Introduction

Potato (Solanum tuberosum) is a worldwide cultivated tuber-bearing plant which is the fourth main food crop in the world after rice (Oryza sativa), maize (Zea mays) and wheat (Triticum aestivum), (Douches et al., 1996). Potato doesnot require special growth conditions; it has been for a long time a major field crop in temperate regions, and increasingly in warmer regions (Haverkort, 1990). In Egypt, potato crop has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato. In addition, the total cultivation of potatoes reached 197,250 feddans which produce 2,039,350 tons of tubers with an average yield of10.34 tons/feddan (Douches et al., 1996). This crop is economically important to Egypt and disturbance in its production affects severely it's local and export impact.

Harmful bacteria or pathogens can affect agricultural crops and produce which results in economical losses (Bathily et al., 2010). *Erwinia carotovora*, a plant pathogen, causes bacterial soft rot in vegetables such as potatoes, cabbages, carrots and many other plants (Altin, N. &Bora, T., 2001). Soft rot is a serious situation in wet and warm areas after harvest. When vegetables are harvested or transported to storage, small cuts and bruises might be inflicted on them. This allows Erwinia carotovorato enter the vegetable and infect it with soft rot, causing it to deteriorate over the next few days. E. caratovora produce extracellular pectinase and breaks down plant tissue by making them osmotically fragile. Signs of soft rot can be seen when the vegetable becomes slimy, mushy and has a darkened area. It also gives off a foul odour. This causes most of the infected crops to be unsuitable for consumption, leading to economical losses as they have to be discarded.

Biological-control treatments consisting of living microorganisms or abiotic products can provide disease protection essentially through one or more of the following: (i) production of antibiotics or other molecules that are deleterious to the pathogen's development, (ii) competition with the pathogen for nutrients and space, or (iii) induced plant resistance. It has more specific effect on the pathogen and has limited impact on the environment (Sigee, 1993). The strategy for biological control of tubers involves the use of antagonistic microorganisms. The bacteria *(Bacillus subtilis* and *Pseudomonas* fluorescent) and the fungi *Trichoderma* viridi. Bacillus subtilis shows biological activity against phytopatogenic bacteria by producing peptide antibiotics (Backman et al., 1997) and reducing the soft rot decaying stored potato tubers (Kloepper et al., 2004). Soil fluorescent and non-fluorescent Pseudomonas spp. have shown biological control of soft rot disease of potato by producing a variety

of secondary antibacterial metabolites including siderophores, antibiotics and surfactants (Compant et al., 2005). Trichoderma Viride is the potential antagonistic fungus which prevents the crops from diseases and able to suppress more than 60 species of pathogens (Abd-El-Khair & Karima, 2007)

In recent years there has been an increasing interest in finding alternatives to bactericides and fungicides. Chemical-control treatments considered as safe, with negligible risk to human health and environment among these strategies, Chitosan (CS) is one of the most abundant biological polysaccharide derived from nature and is obtained by deacetylation of chitin present in crudtaceous. Chitosan is composed of linked 2-deoxy-2-amino-D-glucopyranose units and ß (1→4) linked 2-deoxy-2-acetamido-D-glucopyranose units Figure (1). In spite of its abundance in nature, the commercial utilization of chitosan has been developed only over the last 2 decades; it has emerged as a new biomaterial for food (Rhoades & Roller, *2000*), pharmaceutical (***Illum,*** *1998* and ***Rouget,*** *1859*), medical (Tharanathan & Kittur, 2003 and Utaida etal., 2003), textile (Takai et al., 2002), agricultural (***Dodane & Vilivalam,*** *1998*), and other industries, as well as for waste water purification (***Babel & Kurniawan,*** *2003*).

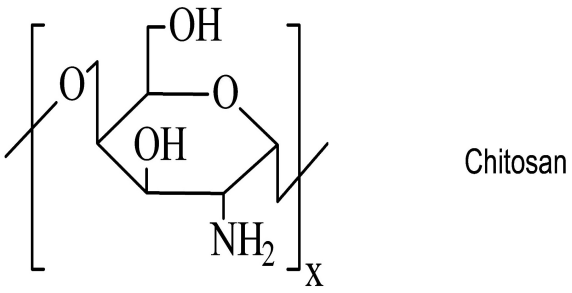


Figure (1): structure of chitosan.

Some satisfactory results have been reported using natural compounds such as chitosan (Muzzarelli, 1983). Chitosan and its derivatives have attracted much attention as antimicrobial agents against fungi, bacteria, viruses and as elicitors of plant defense mechanisms (Chirkov, 2002, Helander et al., 2001and O'Byrne & Booth, 2002). In fact, a number of commercial applications of chitosan benefit from its antimicrobial activity, including its use in food preservation (Rhoades & Roller, *2000*), manufacture of wound dressings (Tharanathan & Kittur, 2003) and antimicrobial-finished textiles (Takai et al., 2002). The lack of understanding of how this industrially valuable biopolymer exerts its antibacterial activities led us to our more systematic study of its mechanism of action. Taking these antecedents into account, the aim of the study reported here was to test the effect of bio-control agents (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma virdi*) individually, biochemical on *Erwinia carotovora* subsp. Followed by testing the effect of combining biochemical and bio-control agent. Then study the effect of this treatment on protein, starch and total sugar content and also define dry matter and specific graphite.

1.1. MATERIALS AND METHODS

1.1.1. Materials

Chitosan (CS) with the deacetylation degree of 70 % with medium molecular weight (Mw) was purchased from Aldrich chemicals.

1.1.2. Potato tuber source

The local farmers provided the fresh maturetubers of potato variety Cara and Spunta. Apparently healthy anduniformly sized tubers were selected for the experiments. Theselected tubers were washed under running tap water and stored in the laboratoryconditions prior to the onset of the experiments.

1.1.3. Isolation and purification of the causal organism

Erwinia carotovora subsp. carotovora was isolated from infected potato tubers, and identified asthe principal pathogen of potato soft rot in storage. E.carotovora cultures were maintained on potato dextrose agar (PDA) slants at 4°C. The spores suspension was prepared from 3-day-old cultures grown at 30°C in nutrient broth medium.After the incubation period the medium was centrifuged at 3000 rpm for 20 min and thepellet was

collected in sterile distilled water (which was considered as bacterial spore andcell suspension) and diluted to a concentration of 6x106 spores/ml (Dodane & Vilivalam, 1998)

1.1.4. Identification of the causal organism

Morphological tests shape of cell and Motility. Physiological and Biochemical tests Cavity formation on crystal violet pectate (CVP) medium and fluorescence on King's B medium were performed as described earlier (Kelman, 1974 and Fang , 1998). The tests used in this study were Gram staining (Gerhardt, 1981), growth in 1-5% NaCl and at 37ºC performed as described in Bergey' s Manual of Systematic Bacteriology (Garrity et al., 1984), Catalase and Nitrate test (Schaad et al., 2001), Strach hydrolysis test (Cowan, 1974), V.P test, Methyl read reaction and H2S production (Bergey's, 1984) and acid production from carbohydrates utilized as source of carbon (Schaad et al., 2001) glucose, maltose, sucrose, lactose, inositol and α-methyl glucoside, amylolysis, utilizationof citrate.

1.1.5. Isolation and identification of Biocontrol agents

Six samples from the rhizospheric soils were taken (up to 10 cmdepth). Samples were placed in sterilepolyethylene bags, closed tightly and stored in the refrigerator at 4°C until use. Samples of each rhizospheric soil were first mixed, suspended in sterile distilled water (1 g in 100 ml) and shaken on rotatory shaker (200 rev/min, 30 min). All treated samples were seriallydiluted up to 106 and spread (0.1 ml) over the surface of nutrient agar (Difco, USA) and soil extract agar (Barakate et al., 2002).When the bacterial colony appeared on the medium, representative isolates werepicked for this study. Pure cultures of biocontrol agent strains were identified using the morphologicaland physiological characteristics according to the methods of (Schaad et al., 2001, Lelliot & Stead, 1987 and Klement et al., 1990).

1.1.6. In Vitro Antimicrobial Activity on Petri Dishes

Antagonistic activity of thebacterial isolates was tested in vitro using plate chloroform method (Wakimoto et al., 1986 and Furuya et al., 1997). Briefly, one loop full of 1-2-day-old probable antagonistic bacterial colony (Bacillus subtili and Pseudomonas fluorescens) grown in yeast peptone dextrose agar YPDA medium was transferred to the center of a petri dish containing 20 mL of YPDA with four replicates of each bacteria. The plates were incubated at 30◦C for2 to 3 days. When the bacteria colonies were formed asseveral millimeters in diameter, the plate was turned upsidedown. A sheet of filter paper was placed in the petridish lid with 0.5mL of chloroform. The dish was kept atroom temperature for 2 h. After complete evaporation of chloroform, 5mL suspension of pathogenic bacteria (ca.108 cfu/mL) was overlaid on each plate. One plate of the soft rot bacterial strain, E. carotovora subsp. Carotovora was used as an indicator bacterium. The plate thus prepared was incubated at 30◦C for 2 days. When an inhibitionzone appeared, its diameter was measured to evaluate theantibacterial activity of the probable antagonistic bacteria (Furuya et al., 1997). The bacterial isolates which showed antagonistic effectagainst indicator bacteria were selected for the furtherstudies.But antagonistic activity of the fungal isolate T.viridi was tested in vitro using disc diffusion method (Furuya et al., 1997). A disc of 5mm in diameter from T.viridi (from 7 days old culture) was placed on the surface of OMAplates seeded with potato scab pathogen. The plates were incubated at 28 ºC for 48 h. The inhibition zone around the discs indicated the antagonistic interaction.

1.1.7. Preparation of different concentration of chemical control chitosan.

Under magnetic stirring 1 g of chitosan mixed with 500 ml of 10 % acetic anhydride the mixture was allowed to stirring in room temperature for 24 hrs to form a homogenous solution. The same procedure was repeated with 3g and 5 g to perepare 3% and 5% respectively.

1.1.8. Tuber inoculation

Prior to inoculation, the tubers were surface sterilized in 10.0% sodium hypochloritesolution for three minutes followed by washing in five changes of sterile tap water.Tubers were allowed to dry at room temperature (about 30°C). After drying, the tuberswounded (except control) by punching 5 holes about 5 mm deep using 2 mm thick nails. Next the tubers were inoculated by submerging in the bacterial suspension for ten minutes. After the inoculation the tubers were allowed to dry at room temperature for thirty minutes prior to the control agent.

1.1.9. Biological and chemical Control of Soft Rot Disease under Storage Conditions.

To evaluate the effectiveness of the selected antagonistic bacteria, fungi and chitosan in reducing soft rot infection in storage potatoes Table 1, fresh tubers of each two potato varieties, Spinta and Cara with six replicates of each treatment were dipped in suspensions of antagonistic bacterium, fungi and chitosan for 30 min and air-dried separately. The treated potato tubers were inoculated with soft rot bacteria E. carotovora subsp. Carotovora by spraying with inoculum suspensions (10^6–10^7cfu/mL).

Table (1): Seventeen treatments were tested on potato plants.

1. Control (non sterilized tuber).		
2. Tuber infestation with E. carotovora		
3. Tuber infestation with CS (1%)	6. Tuber infestation with Bacillus subtilis.	9. Tuber infestation with E. carotovora+Bacillussubtilis + CS (1 %)
		10. Tuber infestation with E. carotovora+Bacillussubtilis + CS (3 %)
		11. Tuber infestation with E. carotovora+Bacillussubtilis + CS (5 %)
4. Tuber infestation with CS (3%)	7. Tuber infestation with Pseudomonas fluorescens	12. Tuber infestation with E. carotovora+Pseudomonasfluorescens + CS (1 %)
		13. Tuber infestation with E. carotovora+Pseudomonasfluorescens + CS (3 %)
		14. Tuber infestation with E. carotovora+Pseudomonasfluorescens + CS (5 %)
5. Tuber infestation with CS (5%)	8. Tuber infestation with Trichoderma virdi	15. Tuber infestation with E. carotovora+Trichodermavirdi + CS (1 %)
		16. Tuber infestation with E. carotovora+Trichodermavirdi + CS (3 %)
		17. Tuber infestation with E. carotovora+Trichodermavirdi + CS (5 %)

Inoculated potato tubers were stored separately atroom temperature.Data on soft rot incidence was recorded after 20 week of inoculation. Numberand weight of soft rot infected tubers were recordedand expressed in percentage using the following formula (Abd-El-Khair and Karima, 2007):

Infection % = number of infected tubers /Total number of tubers ×100.

Loss of weight % =Initialweight−weight after discarding the infected sample /Initialweight ×100

Percentage of disease reduction (PDR) was calculated according to the following formula (Hajhamed et al., 2007):

$$PDR = Ack–Atr/ Ack×100, \quad (2)$$

Where Ack and Atr represent the severity of the disease in control and treated samples, respectively. Total sugar (Dubois et al., 1956), protein content (Thomes & Dutcher, 1924), specific gravity (S.g) (Nissen, 1955), starch content (Simmonds, 1977) and dry weight percentage of tubers (A.O.A.C. 1990).

1.1.10. Data analysis

The collected data were subjected to the analysis of variance and the mean compared either using least significant difference (LSD) or plotting standard error of -means at 5% level of probability (Gomez & Gomez, 1984).

1.2. Results and Discussion

1.2.1. Isolation and Identification of the causal organism

The isolated bacteria identified as Erwinia carotovora subsp. Carotovora according to its morphological and biochemical characters (Table 2). The bacterium was rod shaped with rounded ends, convex, creamy white colonies cells appeared singly and also in pairs. Abd El- Khair and Karima found similar types of colonies (Abd-El-Khair and Karima, 2007). The isolated bacteria were Gram negative .No results were obtained with Starch hydrolysis, Nitrate reduction and V.P test. Positive results were obtained with methyl red reaction, indole formation and bacteria were grew under anaerobic condition. The production of acid from Fructose, Galactose and Glucose were positive results but it was negative from Arabinose, Lactose and Maltose.

(Table 2). Morphological, physiological and biochemical characters of the pathogenic bacterial isolate E. carotovora obtained from naturally infected potato tubers.

Character	Bacterial isolate Od23
Shape of cell	Rod
Motility	+
Gram Reaction	-
Spore forming	+
Growth in 1-5 NaCl	+
Catalase production	+
Yellow pigment	+
Starch hydrolysis	-
Nitrate reduction	-
V.P test	-
Methyl red reaction	+
Indole formation	+
Aerobiosis	+
H2S production	±
Carbon source utilization	
Arabinose	-
Fructose	+
Galactose	+
Glucose	+
Lactose	-
Mannitol	+
Maltose	-

(+) = Positive reaction; (-) = Negative reaction; (±) = Weekly reaction

1.2.2. Characterization and Identification of o biocontrol agents.

Two biocontrol bacteria , *Bacillus subtilis* and *Pseudomonas fluorescens* were identified according to their morphological, cultural and physiological characteristic as stated in Bergey's Manual of Systematic Bacteriology, *Bacillus subtilis* was rod ,long, grame positive , sporulating , motile, negative in Pigmentation and soft rot . The tested isolate *Bacillus subtilis* resulted positive reactions with fructose, sucrose, glucose, galactose, maltose, dextrose, glycerol, starch hydrolysis, gelatin liquefication, V.P. Test and Catalase activity. Negative reactions with mannitol, arabinose, lactose, menthol, raffinose and Indole formation. *Pseudomonas fluorescens* was rod, short, grame negative, non-sporulating and motile, negative in soft rot. the tested isolate *Pseudomonas fluorescens* resulted positive reactions with fructose, glucose, maltose , glycerol and Catalase activity . Negative reactions with Mannitol, sucrose,arabinose, , galactose, lactose, dextrose, menthol, raffinose, starch hydrolysis ,gelatin liquefication , Indole formation and V.P. Test (Table 3).

On the basis of the above physiological, biochemical, and carbon sources utilization test results, the antagonistic bacterial were identified as a member of the genus *Bacillus subtilis* and *Pseudomonas fluorescens*. The fungal biocontrol agent *Trichoderma viridi* was identified according to its morphological, cultural characteristic. Conidial wart is conspicuous and Conidiophores are Irregularly branched; branches usually not paired on PDA after incubation at optimum temperature for 48 h (Gams % Bisset, 1998 and Rifai, 1969).

(Table 3). Characterization and identification of two bacterial strains Pseudomonas fluorescens and Bacillus subtilis.

Character	Pseudomonas fluorescens	Bacillus subtilis
Shape of cells	Rod	Rod
Size	Short	Long
Gram's staining	-	+
Sporulation	-	+
Motility	+	+
Pigmentation	+	-
Soft rot	-	-
Growth in 1- 5%	+	+
Utilization of sugar:		
Mannitol	-	-
Fructose	A	A
Sucrose	-	A
Arabinose	-	-
Glucose	A	A
Galactose	-	A
Lactose	-	-
Maltose	A	A
Dextrose	-	A
Glycerol	A	A
Menthol	-	-
Raffinose	-	-
Physiological characteristics		
Starch hydrolysis	-	+
Gelatin liquefication	-	+
Indole formation	-	-
V.P. Test	-	+
Catalase activity	+	+
Oxidase reaction	-	-

(+): Positive reaction or growth, (-): Negative reaction or no growth, (A): Acid

1.2.3. In Vitro antimicrobial and chemical activity on petri dishes.

All biocontrol agents, Bacillus subtilis, Pseudomonas fluorescens, Trichoderma virdi and chemical control Cs by concentration 1, 3, 5% were significantly superior in inhibiting the growth of Erwinia carotovora subsp. Carotovora (Table 4). Trichoderma virdi showed highest inhibition zone (26.3 mm) after 48 hours of

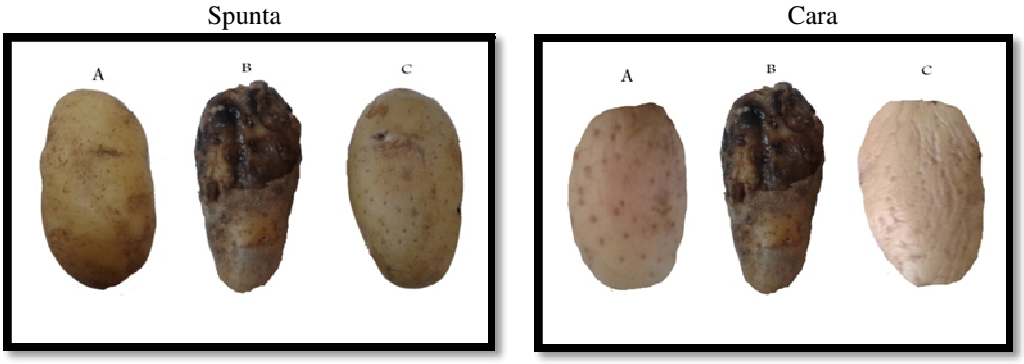
incubation followed by Bacillus subtilis with inhibition zone (23.3 mm) and Pseudomonas fluorescens with inhibition zone (22.0 mm) after 48 hours of incubation (Table 4). Long et al. reported that the genus Bacillus and Pseudomonas have antagonistic activity against various plant pathogenic bacteria including soft rot bacterium E. carotovora subsp. carotovora in vitro (Long et al., 2003). Abd El-Khair and Karima reported that Trichoderma has antagonistic activity against E. carotovora subsp. carotovora in vitro (Abd-El-Khair and Karima, 2007). Cs showed highly inhibition zone with concentration 5% (28.6 mm) followed by Chitosan with concentration 3% (25.3 mm) and Chitosan with concentration 1% (22.9 mm) after 48 hours of incubation (Raju et al., 2006 and Hadwiger & Lee, 2013).

Table (4): Comparative efficiency of biocontrol agents and Chitosan against Erwiniacarotovorasubsp. Carotovora.

Antagonism	Inhibition zone in mm
Bacillus subtilis	23.3
Pseudomonas fluorescens	22.0
Trichodermavirdi	26.3
Chitosan 1%	22.9
Chitosan3%	25.3
Chitosan5%	28.6

1.2.4. Biological and chemical Control of Soft Rot Disease under Storage Conditions.

All tested bio control agents have significantly reduced severity of bacterial soft rot disease of potato individually or combined with Cs compared with the control. The highest severity of disease completely decreased by the treatment Cs 5% with biocontrol agents (T. virdi , B. subtilis and P. fluorescens respectively) each after 20-week storage potatoes of two Spunta and Cara varieties by (91,8 , 83.6 and 80.3% respectively in Spunta c.v) and (88.6, 84.1 and 79.3% respectively in Cara c.v) as show in Figures (2,3 and 4). Followed by treatment chitosan 3% with biocontrol agents (T. virdi , P. fluorescens and B. subtilis respectively in Spunta c.v) by (88.6, 80.4 and 76%respectively in Spuntac.v) and (85, 80.1 and 77.3% respectively in Cara c.v) and treatment chitosan 1% with biocontrol agents (T. virdi , P. fluorescens and B. subtilis respectively) each by (75.3,71.3 and 66.4% respectively in Spunta c.v) and (69.2, 65.5 and 62.4% respectively in Cara c.v) compared with T. virdi , B. subtilis and P. fluorescens individually as show in Figure (5).



Figure(2): Effect of Chitosan 5% and T. virdi on potato tubers (Spunta and Cara c.v). A: Healthy tuber (Untreated control); B: Tuber infected with E.carotovorasubsp. Carotovora; C: Tuber infected with E. carotovora subsp. Carotovora + Chitosan 5%+ T. virdi

Spuntac.v Cara c.v

Figure (3): Effect of Chitosan 5% and B. subtilis on potato tubers (Spunta and Carac.v). A: Healthy tuber (Untreated control).; B:Tuber infected with E.carotovorasubsp. Carotovora; C: Tuber infected with E. carotovora subsp. Carotovora + Chitosan 5%+ B. subtilis.

Spuntac.v Cara c.v

Figure(4): Effect of Chitosan 5% and P. fluorescens potato tubers (Spunta and Cara c.v). A: Healthy tuber (Untreated control).; B:Tuber infected with E.carotovorasubsp. Carotovora; C: Tuber infected with E.carotovorasubsp. Carotovora + Chitosan 5%+ P. fluorescens

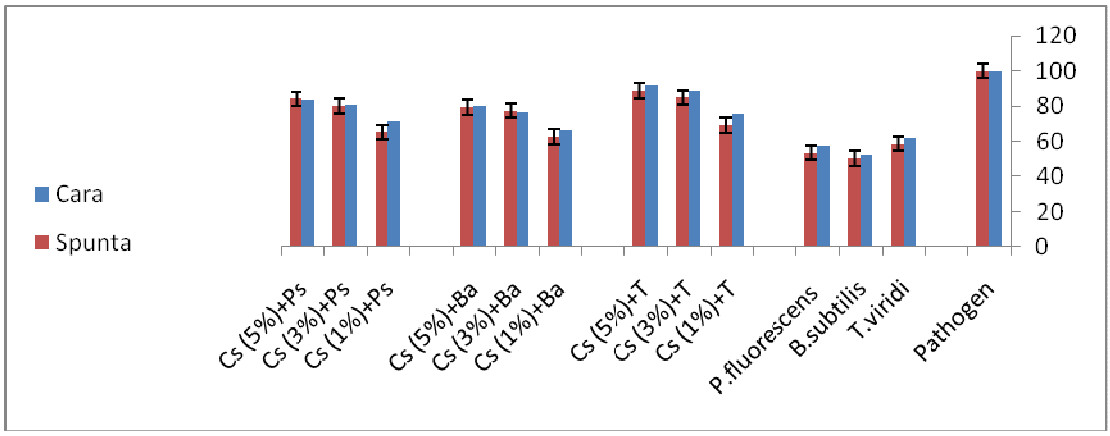


Figure (5): Effect of biocontrol agent and chitosan on the severity of potato tuber soft rot disease. Bars indicate the standard error.

Total sugar, protein content , starch content ,specific gravity(S.g) of tubers were completely increased by the treatment Cs 1% , Cs 3% and the highest result with Cs 5% each with biocontrol agents (T. virdi , B. subtilis and P. fluorescens respectively) of two Spunta and Cara varieties compared with T. virdi , P. fluorescens and B. subtilis individually and control . The results are shown in Figures (6, 7, 8 and 9). And percentages losing of weight were decreasing by the pathogen as showen in figure (10).

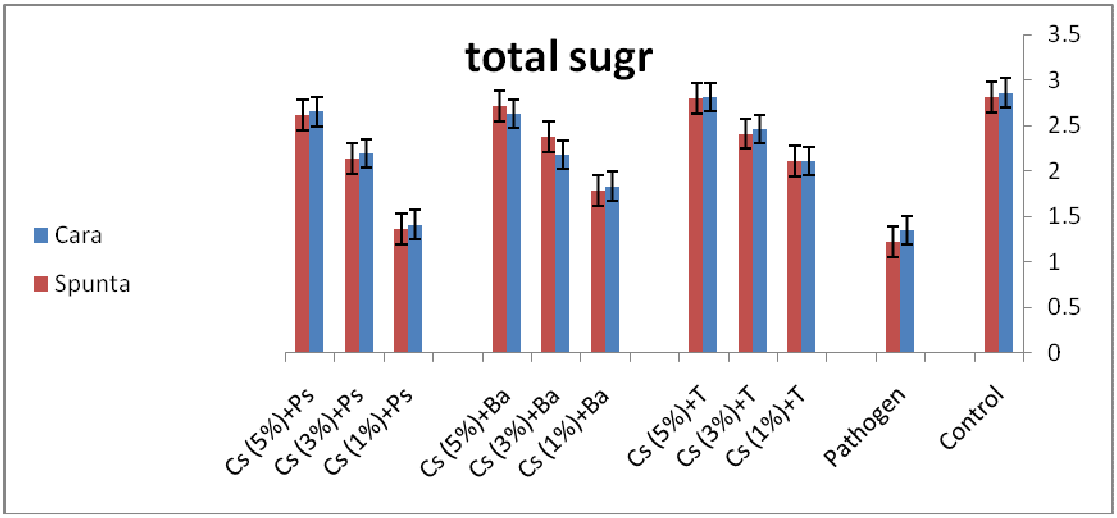


Figure (6): Effect of biocontrol agents and chitosan on Total sugar of tubers. Bars indicate the standard error.

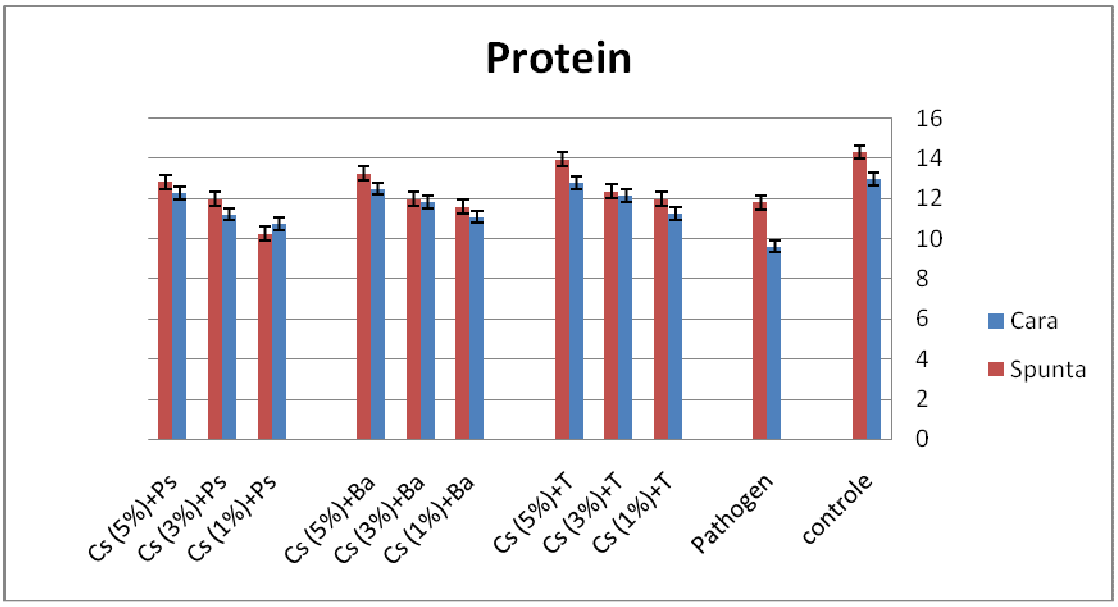


Figure (7): Effect of biocontrol agents and chitosan on protein content of tubers. Bars indicate the standard error.

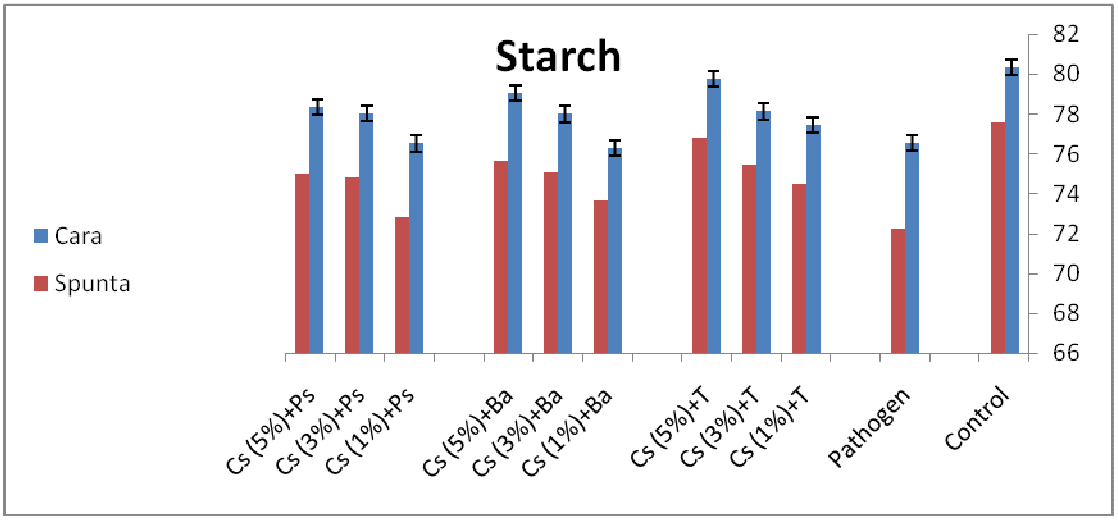


Figure (8): Effect of biocontrol agents and chitosan on starch content of tubers. Bars indicate the standard error.

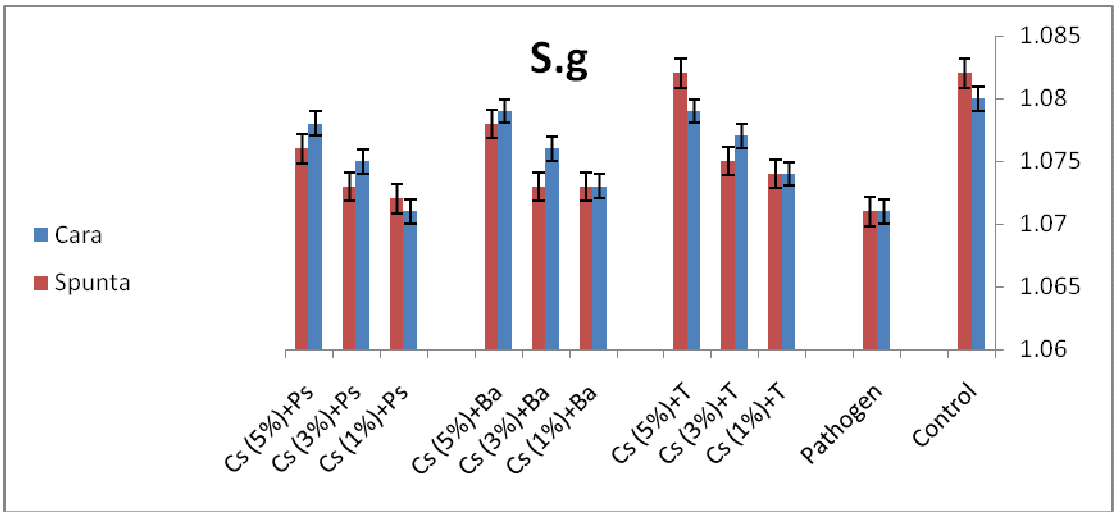


Figure (9): Effect of biocontrol agents and chitosan on specific gravity of tubers. Bars indicate the standard error.

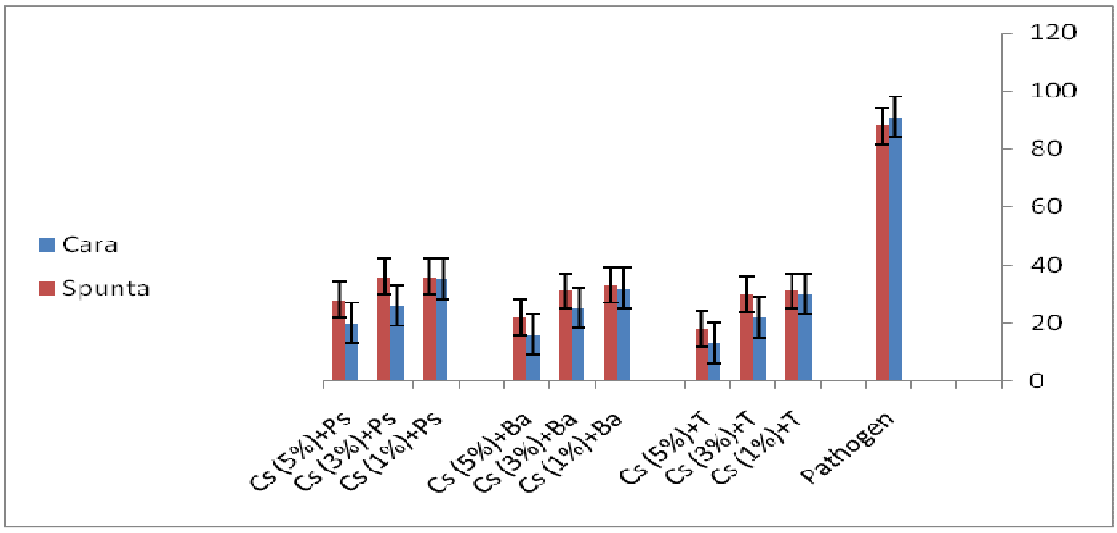


Figure (10): Effect of biocontrol agents and chitosan on Losing weight % of tubers. Bars indicate the standard error.

The results of the study demonstrated that the identified antagonistic bacterial (T. virdi, B. subtilis and P. fluorescens) combined with Cs 5% can significantly inhibit the growth of soft rot bacteria *in vitro* and in storage. Trichoderma viride was reported by several workers as the best antagonists for growth inhibition of several plant pathogen.It has various mechanisms of bio control include antibiosis, parasitism, inducing host-plant resistance, and competition confrontation with fungi (Howell, 2003), fungal cell wall degrading enzymes (Sharma et al., 2009 and Chutrakul et al., 2008). Fluorescent pseudomonads are aggressive rhizosphere colonizes and produce a wide range of antimicrobial compounds (Gross, 1988). Certain fluorescent pseudomonads can protect plants from diseases caused by root pathogens and often this biocontrol effect involves antimicrobial compounds such as siderophores (Bakker et al., 1987), hydrogen cyanide (Voisard et al., 1989) and antibiotics (Raaijmakers et al., 2002) like phenazine-1-carboxylate, pyroluteorin and 2,4-diacetyl phoroglucinol..Bacillus sp. was considere one

of the best antagonistic bacteria for limiting growth of many pathogens demonstrated strong antagonistic actions against the soft rot bacterial pathogen which was previously identified as the most virulent strain of *Erwiniacarotovora*subsp. *carotovora* both in *invitro* and under storage conditions. Many researchers previously exploited *Bacillus* sp. to control soft rot bacteria in various plants (Abd-El-Khair and Karima, 2007 and Sharga & Lyon, 1998).

Conclusion:

The three treatment of biocontrol agents combined with chitosan 5% were showed the highest level of antibiosis against pathogenic organism (*Erwinia carotovora*) the causal organism of potato soft rot, and achieve a significant control of disease on the tested varieties Cara and Spunta.

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