Research Journal of Agriculture and Biological Sciences, 3(5): 463-473, 2007

Application of Some Bactericides and Bioagents for Controlling the Soft Rot Disease in Potato

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Abstract: The bactericides, i.e., streptomycin sulfate, Starner and Micronite Soreil and two bioagents, Tricoderma *harzianum* and *Bacillus subtilis* were applied for controlling the soft rot disease causing by *Erwinia carotovora* subsp. *carotovora in vitro* and in field. *In vitro*, results showed that the Starner, *B. subtilis* and *T. harzianum* reduced the pectolytic enzymes (PG and PME enzymes), while Starner and streptomycin sulfate reduced the cellulolytic enzyme (Cx). The tested materials were also powerful bactericide against the bacterial soft rot pathogen. Streptomycin sulfate, *T. harizanum* and *B. subtilis* prevent the soft rot disease in daughter potato tubers and increased the vegetative characters, plant height and number of leave per plant. Results show that plant tubers yield and the average of tuber weight has been increased when the above bactericides were applied, comparing with un-treated plants. Starner and Micronite Soreil gave a moderate effect in reducing the incidence of soft rot disease, while a positive effect on tuber weight and plant tuber yield has been recorded than control. The incidence of soft rot disease and the weight loss in potato tubers resulting from treated plants studied in storage.

Keyword: *Erwinia carotovora* subsp. *carotovora,* Potato, Control, *Trichoderma harzianum, Bacillus subtilis*, Streptomycin sulfate, Starner, Micronite Soreil, Application, Enzymes, Bacterial count.

INTRODUCTION

Erwinia carotovora subsp. *carotovora* (Jone) Dye is the major disease pathogens affecting potato seed tuber pieces after cultivation, during vegetative growth and on potato tubers during storage[1, 2]. The soft rot is considered as one of the limiting factors on potato production in the world as well as in Egypt[1]. The pathogen can rot tubers in store or in the filed where early decay of seed tuber pieces can result in non-emergence or blanking[3]. When the rotted mother tubers could emerged infection of the stems can be occurred[4].

The maceration process involves the depolymerization of the pectin of plant cell walls and the middle lamella. Pectin is a hetero-polysaccharide with a backbone consisting of partially esterified galacturonic acid. The enzymes of pectinases secreted by plant pathogens of soft-rot bacterium *Erwinia carotovora*, as part of their strategy for penetrating the plant host cell walls. The production of pectinase (poly-lacturonase), the major virulence determinant of soft-rot *Erwinia* species, is controlled by many regulatory factors[5]. The crude extracts of soybean seeds were added to the growth medium of *E. carotovora* subsp. *carotovora*, the population was substantially checked and the total pectolytic and cellulolytic enzyme activities were decreased, but to a lesser extent than growth[6]. Streptomycin sulfate (90 %) and tetracycline hydrochloride (10 %) [Streptocycline] recorded the maximum growth inhibition zone of 27. 66 mm. The maximum inhibition of pectinlyase (PL); polygalacturonase (PG) and protopectinase production were recorded by the same antibiotic. The antibiotics had a significant effect on the production and activity of cell wall degrading enzymes produced by plant pathogenic microorganisms[7]. Benzoic acid and sodium benzoate were effective in controlling the soft rot diseases in both tomato fruits and potato tubers[8]. Ethylene diamine tetracetate (EDTA) and the antibiotic nisin were inhibited the growth of pectolytic soft –rotting bacteria[9].

Disease incidence of soft rot disease can be reduced by antibacterial treatments of seed tubers in field application[10]. Benzoic acid and sodium

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benzoate at 1, 5 and 10 mM inhibited the growth of *E carotovora* subsp. *carotovora* in potato tubers[8]. Pre-sowing applications of stable bleaching powder and sterptocycline were effective for preventing soft rot pathogen, sproutng and weight loss of potato tubers[11]. Soil drenching with stable bleaching powder at 10 Kg / ha gave better control of *E. carotovora* than sprays of strptocycline and Blitox 50 (copper oxychloride)[12]. Treatment of potatoes with bioagents before planting in soil infested with *E. carotovora* reduced soft rot severity in daughter potato tubers[13, 14]. The number and weight of tubers increased when potato plants were treated with bioagents. A *Bacillus* strains produced a natural biocontrol agents, which can be used as biopesticides against spoilage microorganisms[15].

The objective of this work aimed to study the role of some bactericides and bioagents in decreasing the softening tubers in field production and in minimizing the existence of the initial inoculum potential of soft rot pathogen associated the potato tubers pre-storage.

MATERIALS and METHODS

Plant Material: Potato tubers (cv. Spunta) were planted in National Research Centre (NRC) farm at El-Kanater El-Kheriya, Kalubiya governorate, Egypt, during January of 2006 season. Tuber pieces contained one or more sprouts, were cut carefully from each tuber and were used in sowing.

Antibacterial Materials:

Bactericides: Streptomycin sulfate (El-Nasr Pharmaceutical Chemicals Co. Egypt., Starner (oxalinic acid, 20% WP, Sumitomo Chemicals Co. Japan); and Micronite Soreil 70 % WP (Sulfur) were used dressing.

Bioagents: *Trichoderma harzianum* and *Bacillus subtilis* which proved to be highly antagonistic effect against phytopathogens[16, 17], were used as seed dressing.

Soft Rot Pathogen: Soft rot pathogen was detected in seed potato tubers as recommended by Perombelon[18]. Unwashed seed tubers were placed individually in plastic bag with 20 mL of distilled water, then bags sealed and incubated at 18 ºC for 10 – 15 days. Soft rot symptoms were recorded[19, 20]. For isolation soft rot pathogen (*Erwinia carotovora* subsp. *carotovora*); potato tubers showing soft rot symptoms were used. The bacterial pathogen was isolated using Nutrient glucose (2%) agar medium (NGA) [3 g beef extract; 5 g peptone; 20 g glucose; 15 g agar and 1000 ml distilled water. PH, 7. 2][21]. The isolated bacteria was identified according to pathological, morphological, cultural, physiological and biochemical characters[20].

Media and Growth Conditions: *E. carotovora* subsp. *carotovora* cells were grown in Nutrient-broth medium [3 g beef extract; 5 g peptone; 20 g glucose; and 1000 ml distilled water. PH, 7. 2] (NBM)[20]. Bacterial cells were incubated at 30 ^{0}C for 48 h. The bacterial culture were used for *in vitro* tests.

***In vitro* Tests:** The efficacy of bactericides and bioagents were tested at two concentration (first and second spray) against enzymatic activities and population of *E. carotovora* subsp. *carotovora* cultural medium. Streptomycin sulfate at concentrations of 25 and 50 ppm; Starner at concentrations of 25 and 50 ppm and Micronite Soreil at concentrations of 1 and 2 % were tested. *T. harzianum* and *B. subtilis* grown in NBM separately at 30 ºC for 48 h, then each bioagent filters were collected by filtering though sterile 0. 45 µ membrane filter (cellulose nitrate, Whatman)[22]. Each bioagent filter was tested at concentrations of 2 and 4 %.

Enzymes Activity: Pectolytic and celluloytic enzymes of *E. carotovora* subsp. *carotovora* pathogen were determined by the methods described Ech andi *et al.*[23] and MacMillan and Voughin[24]. The production of pectic enzymes; polyglacturonase (PG) and pectin methyestrase (PME) were carried out using the medium (4. 6 g citrus pectin, 5. 0 g yeast extract, 5. 0 g peptone and 5. 0 g K2HPO4)[24]. Also, the same medium supplemented with 4. 6 g carboxymethyl cellulose (CMC) instead of pectin was used for the production of cellulolytic (Cx) enzymes. Flasks contained 50 ml of the medium were autoclaved. Each tested treatment was added the medium flask to obtain the tested concentrations. Three flasks were used as control. Each treated flasks were inoculated with 0. 5 ml of *E. carotovora* subsp. *carotovora* suspension (10^{7-9} colony forming unit (cfu) / ml). After incubation at 30 ^{0}C for 72 h, the supernatants were obtained by centrifugation at 5000 rpm for 20 min, then the supernatants (crude enzymes preparations) were used for enzymatic assay.

PG Assay: PG activity was assayed by estimating the loss viscosity of 1. 2% citrus solution after incubation at 30 ^{0}C [23]. Reaction mixture consisted of 5 ml crude enzyme + 5 ml of 1. 2 % pectin solution buffered at pH 4. 5 with phosphate buffer. Boiled crude enzymes were used for control.

PME Assay: PME activity was determined by the titration method using 0. 01 N NaOH solution after incubation for 24 h at 30 ^{0}C[25]. Reaction mixture consisted of 5 ml crude enzyme + 20 ml of 1. 5% pectin solution (pH 7. 0). Activity was expressed as milliliters of NaOH solution required to neutralize the carboxylic groups.

Cx Assay: Cx activity was determined by measuring the loss in viscosity of 1% carboxymethyl cellulose solution, after incubation for 3 h at 30 ^{0}C [25]. Reaction mixture (5 ml of crude enzyme + 5ml of 1% CMC, pH 5. 0) were used. Boiled crude enzymes were used as control.

Population of *E. Carotovora* Subsp. *Carotovora*: Each tested treatment, separately, was added to the autoclaved NBM (20 ml) to obtain the tested concentration. Then, the flasks of medium were inoculated with 0. 5 ml bacterial suspension (10 $^{7-9}$ cfu / ml). Each treatment as control was used. The inoculated flasks were incubated at 30^{0}C for 72 h. Population of the *E. carotovora* subsp. *carotovora* in growth medium treated with the tested bactericides and bioagent filters were determined using diluted method and pour plate technique [26]. One ml of bacterial culture was diluted in 99 ml sterile water. Then, serial dilutions form 10^{-3} to 10^{-7} were prepared. Population count were measured on NGA medium. Bacterial counts were expressed as colony forming unit (cfu) per milliliter (ml).

Field Experiment: The experiment was designed in a randomized complete block, three lines in each block were used as a replicates for each treatment, where each line include 12 pits and one seed piece was sown in each pit. Irrigation and fertilization were carried out as recommended[27].

The efficacy of streptomycin sulfate at concentration of 200 ppm. ; Starner at concentration of 200 ppm. ; and Micronite Soreil 70 % WP (Sulfur) at concentration of 1 % were used as seed tuber pieces dressing in field application against *E. carotovora* subsp. *carotovora* pathogen[28]. Seed tuber pieces were treated with each bactericide concentration, separately, for 5 min before sowing.

The efficacy of bioagents of *Trichoderma harzianum* (3 X 10^8 propgules / ml) and *Bacillus subtilis* (3 X 10^8 cfu / ml), which proved to be highly antagonistic against phytopathogens[16, 29, 17], were used as seed pieces dressing against soft rot pathogen in field application. Mixture of each bioagent suspension was mixed, separately, with seed pieces for 5 min. Then, treated seed pieces were sown.

Soft Rot Incidence:

At harvest: Percentage of softening potato tubers, for each experimental treatment, was recorded using the following formula:

$$\text{Infection\%} = \frac{\text{No. of infected tubers}}{\text{Total tuber no.}} \times 100$$

At storage: Samples of harvested potato tubers (no soft rot symptoms occurred) were collected from each field application treatments and stored separately for 3 months under natural conditions. Stored potato tubers were examined for presence of soft rot symptoms through the storage period. Percentage of softening tubers, for each treatment, was calculated at the end of storage period. Loss of weight of tubers also, for each treatment, in the end of storage period was calculated as percentage using the following formula:

$$\text{Loss of weight \%} = \frac{\text{Weight before storage - weight after storage}}{\text{Weight before storage}} \times 100$$

Vegetative Growth and Yield Characters: A random sample of nine plants were taken 70 days after planting from each treatment to determent the average of stem length (plant height) and average number of leaves per plant[30]. After harvest, tuber samples of nine plants from each experiential treatment were collected individually. Then, average tuber weight (g), average tuber number per plant and total yield (Kg) per plant were determined[27].

Statistical Analysis: The statistical analysis was done according to Steel and Torrie[31]. Normal F test were used and the means were compared by L. S. D. at level of significant.

RESULTS AND DISCUSSIONS

Bacterial Soft Rot Pathogen: The bacterial isolates, which isolated from potato seed pieces, were pathogenic to potato tubers under artificial infection conditions. The morphological characters of bacterial isolates were Gram negative and short rods. The cultural character of bacterial colonies was creamy white in color, circular, convex, smooth, opalescent, butyrous and entire margin. The isolated bacteria identified as *Erwinia carotovora* subsp. *carotovora* according to pathological, morphological, cultural and biochemical characters[32].

Table 1: Characteristics of *Erwinia carorovora* subsp. *carotovora* from potato

Tests	Reaction
Soft rot on potato slices	+
Gram staining	G+
Yellow colonies on YDC medium	-
Fluorescent pigment on King s B medium–	
Deep pits on CVP medium	+
Anaerobic growth.	+
Gelatin liquefaction.	+
Growth at NaCL 5 %.	+
Growth at 37^{0}C	+
Sensitivity to Erytheromycin.	-
Acid from:	
Arabinose.	+
Trehalose.	+
Glucose.	+
Lactose.	+
Mannitol.	+
Salicin.	+
Starch.	+
Gas from glucose.	+

Data in Table (1)summaries the important characters of *E. carotovora* subsp. *carotovora*. The bacterial isolates grew at anaerobic growth conditions, sodium chloride(5%), temperature of 37^{0}C, could gelatin liquefaction and sensitive to erythromycin. The bacterial isolates produced acid only from arabinose, trehalose, maltose, lactose, mannitol, slaicin and starch except glucose producing acid and gas. These results agree with those recorded by Perombelon[18]. He reported that the commercial seed potato stocks can be contaminated with *E. carotovora* subsp. *carotovora* pathogen. He suggested that the potato seed itself is the major source of *E. carotovora* subsp. *carotovora* for the growing crop causing the soft rot disease in and in storage. Therefore, It is be very important reduced the bacterial count of soft rot pathogen on potato seed surface, where the pathogen infected plant, rotting mother and daughter tubers [18].

***In vitro* Tests:**

The Efficacy of Bactericides and Bioagents On:

Pectolytic and Celluloytic Enzymes Activities: The efficiency of two tested concentrations of streptomycin sulfate (25 & 50 ppm), Staner (25&50ppm), Micronite Soreil (1 & 2%), *B. subtilis* filters (2 & 4%) and *T. harzianum* filters (2 & 4 %) on the ability *E. carotovora* subsp. *carotovora* to secrete PG, PME and Cx enzymes *in vitro* testes are shown in Table (2).

Pg Enzyme Activity: Activity of PG enzyme, was assayed by estimating the relative loss in viscosity of 1. 2% pectin citrus solutions, as shown in Table (2). Data cleared that the PG enzyme yield of *E. carotovora* subsp. *carotovora* in treated cultural medium was less than untreated culture. The inhibitory effect of bactericide and bioagent treatments on PG enzyme activity of *E. carotovora* subsp. *carotovora* was increased by increasing the concentration of tested material**.** There were significant differences observed between the inhibitory effect of tested treatments, between the effect of concentrations and between the incubation periods. After 48 h of incubation at 30 ^{0}C, the strongest inhibition of PG enzyme secretion was obtained with Starner, followed by *B. subtilis, T. harzianum*, streptomycin sulfate and Micronite Soreil, where the relative loss in viscosity values were 7. 6, 7. 8, 12. 9, 13. 5 and 15. 8 % at the first concentration, respectively. The PG reduction (%) were 55. 8, 54. 7, 25. 0, 21. 5 and 8. 1 % comparing the control, respectively. At the second concentration, *B. subtilis* gave the highest inhibition of PG activity, followed by Starner, *T. harzinaum*, Micronite Soreil and streptomycin sulfate, where the relative loss in viscosity values were 6. 2, 6. 4, 6. 4, 9. 8 and 10. 1 %, while the reduction values enzyme activity comparing the control were 64. 0, 62. 8, 62. 8, 43. 0 and 41. 3 %, respectively (Table, 2).

After 72 h of inhibition, the best PG enzyme inhibitory effect obtained with *T. harzinum*, Starner, streptomycin sulfate*, B. subtilis* and Micronite Soreil at the first concentration, respectively. The values of relative loss in viscosity were 12. 5, 14. 0, 15. 0, 15. 3 and 17. 0 %, while the values of enzyme reduction were 33. 9, 25. 9, 20. 6, 19. 5 and 10. 1 %, respectively. At the second concentration, the highest inhibition of PG enzyme yield obtained with Starner, followed by *B. subtilis, T. harzianum*, streptomycin sulfate and Micronite Soreil, respectively. The values of relative loss in viscosity were 8. 6, 9. 7, 10. 6, 11. 0 and 11. 0 %, while the PG enzyme reduction were 54. 5, 48. 7, 43. 9, 41. 8 and 41. 8 % comparing the control, respectively (Table, 2). Our results suggested that the bactericide and bioagent treatments were the most effective to inhibit of the PG enzyme activity after 48 h of incubation comparing with their effects after 72 h of incubation as well as the control. Especially. *B. subtilis T. harzianum* and Starner. The results revealed that the tested treatments gave the highest inhibition of PG enzyme activity when applied the high concentration in culture medium.

PME Enzyme Activity: PME enzyme activity of *E. carotovora* subsp. *carotovora* at tested concentrations of bactericide and bioagent treatments, by titrating with 0. 01 N NaOH solution to neutralize the carboxylic group produced from 1. 5 % pectin citrus solution, after 48 and 72 h of incubation are shown in Table (2). Data showed that the tested treatments reduced the PME enzyme activity comparing the control

Table 2: Pectolytic and celluolytic enzyme activities of *Erwinia carotovora* subsp. c*arotovora* resulting as reaction to bactericides and bioagent treatments (*in vitro*).

Treatments		Enzymatic activities											
		Pectolytic								Cellulolytic			
		PG				PME				Cx			
		48h		72h		48h		72h		48h		72h	
	Con.	Visc. %	Red. %	Visc. %	Red. %	Visc. %	Red. %	Visc. %	Red. %	Visc. %	Red. %	Visc. %	Red. %
Bactericides:													
Streptomycin S.	25ppm	13. 5	21. 5	15. 0	20. 6	2. 1	16. 0	2. 2	26. 7	8. 5	42. 2	9. 3	48. 3
	50 pm	10. 1	41. 3	11. 0	41. 8	1. 7	32. 0	2. 1	30. 0	1. 8	87. 8	3. 8	78. 9
Starner	25ppm	7. 6	55. 8	14. 0	25. 9	1. 1	56. 0	1. 9	36. 7	7. 4	50. 0	9. 2	48. 9
	50ppm	6. 4	62. 8	8. 6	54. 5	0. 5	80. 0	0. 4	86. 7	1. 5	89. 8	4. 1	77. 2
Micronite S.	1%	15. 8	8. 1	17. 0	10. 1	1. 3	48. 0	1. 9	36. 7	13. 6	7. 5	16. 4	8. 9
	2%	9. 8	43. 0	11. 0	41. 8	1. 0	60. 0	1. 8	40. 0	3. 2	78. 2	5. 4	70. 0
Bioagents:													
B. subitlis	2%	7. 8	54. 7	15. 3	19. 5	1. 6	36. 0	1. 8	40. 0	13. 0	11. 6	13. 6	24. 4
	4%	6. 2	64. 0	9. 7	48. 7	0. 7	72. 0	0. 7	76. 7	7. 9	46. 3	11. 1	38. 3
T. harzianum	2%	12. 9	25. 0	12. 5	33. 9	0. 6	76. 0	0. 8	73. 0	7. 0	52. 4	13. 2	26. 7
	4%	6. 4	62. 8	10. 6	43. 9	0. 5	80. 0	0. 6	80. 0	5. 5	62. 6	12. 0	33. 3
Control		17. 2	-	18. 9	-	2. 5	-	3. 0	-	14. 7	-	18. 0	-

LSD 0. 05 (PG – PME – Cx):
Treatments (T) = 0. 34 – NS – 0. 34 Concentrations (C) = 0. 26 -1. 76 – 0. 26 T X C = 0. 58 – NS- 0. 60
Incubation (I) = 0. 22 -NS – 0. 22 T X I =0. 26 –NS – 0. 50 C X I = 0. 38 – NS -0. 38
TXC X I =0. 84 – NS -0. 84

treatment. There no significant differences observed between the inhibitory effect of bactericide and bioagent treatments and between the effect of incubation periods, while the significant difference recorded between the tested concentrations. After 48h of incubations (1stconcentration), the highest inhibition of PME enzyme activity obtained with *T. harzianum*, followed by Starner, Micronite Soreil**,** *B. subtilis* and streptomycin sulfate, where the average of milliliters of 0. 01 N NaOH were 0. 6, 1. 1, 1. 3, 1. 6 and 2. 1%, respectively. These treatments reduced the PME enzyme yield about 76. 0, 56. 0, 48. 0, 36. 0 and 16. 0 % comparing with the control treatment (Table, 2). At 2^{ed}concentration, the less milliliters of NaOH were obtained with *T. harzianum* (0. 5ml), followed by Starner (0. 5ml)*, B. subtilis* (0. 7ml), Micronite Soreil (1. 0 ml) and streptomycin sulfate (1. 7ml), respectively. The PME enzyme activity was reduced about 80. 0, 80. 0, 72. 0, 60. 0 and 32. 0 %, respectively.

After 72 h of incubation (1st concentration), the *T. harzianum, B. subtilis*, Micronite Soreil, Starner and streptomycin sulfate reduced the PME enzyme activity, where the values of NaOH solution were 0. 8, 1. 8, 1. 9, 1. 9 and 2. 2 ml, while the enzyme reduction (%)were 73. 0, 40. 0, 36. 7, 36, 7 and 26. 7 comparing the control, respectively. At 2^{ed} concentration, the Starner, *T. harzianum, B. subtilis*, Micronite Soreil and strepto-mycin sulfate reduced the PME enzyme yield comparing the control, respectively (Table, 2), where the required milliliters of NaOH solution were 0. 4, 0. 6, 0. 7, 1. 8 and 2. 1, respectively. The values of PME activity reduction were 86. 7, 80. 0, 76. 7, 40. 0 and 30. 0 %, respectively. It is revealed that the lower milliliters of NaOH, parallel the lowest in PME activity (Table, 2).

Cx Enzyme Activity: The efficiency of bactericide and bioagent treatments on the ability of *E. carotovora* subsp. *carotovora* to secrete the Cx enzyme, were expressed as the percentage of relative loss in viscosity of CMC solution, are shown in Table (2). After 48h of incubation period at 30 ^{0}C, the relative loss in viscosity values were 7. 0, 7. 4, 8. 5, 13. 0 and 13. 6 % with *T. harzianum*, Starner, streptomycin sulfate, *B. subtilis* and Micronite Soreil at the first concentration, respectively. The values of Cx enzyme yield reduction

Table 3: Effect of bactericides and bioagent filters on the population *of E. carotovora* subsp. *carotovora* in culture medium.

		Count of *E. arotovora subsp. crotovora* (10^7)					
		24h		48h		72h	
Treatments	Con.	Count (cfu / ml)	Reduction %	Count (cfu / ml)	Reduction %	Count (cfu / ml)	Reduction %
Bactericides:							
Streptomycin S.	25 ppm	1. 7	79. 5	2. 3	36. 2	2. 3	73. 3
	50 ppm	0. 8	90. 4	1. 2	85. 5	1. 2	86. 1
Starner	25 ppm	2. 7	67. 5	3. 7	55. 4	4. 7	45. 3
	50 ppm	1. 3	84. 3	2. 0	75. 9	2. 3	73. 3
Micronite S.	1%	7. 7	7. 2	7. 9	4. 8	7. 9	8. 2
	2%	3. 6	56. 6	5. 8	30. 1	5. 7	33. 7
Bioagents:							
B. subitlis	2%	1.2	85.5	1.8	78. 3	2. 0	76. 7
	4%	0. 5	94. 0	0. 8	90. 4	1. 1	87. 2
T. harizanium	2%	4. 0	51. 8	5. 8	30. 1	5. 5	36. 1
	4%	0. 7	91. 6	1. 4	83. 1	1. 5	82. 6
Control		8. 0	-	8. 3	-	8. 6	-

LSD 0. 05 (bacterial count)
Treatments (T) = 3. 9 Concentrations (C) = 3. 0 T X C = 6. 8 Incubation (I) = 3. 0
TX I = NS C X I = NS TXC X I = NS

Table 4: Percentage (%) of softening tubers and loss of tubers weight resulting bactericide and bioagent treatments.

	Softening	After three months of storage of:	
Treatments	tubers %	Softening tubers[1] (%)	Loss of tubers weight[2] (%)
Bactericides:	-	-	-
Streptomycin S.	00. 0	33. 3	48. 7
Starner	11. 0	25. 0	62. 1
Micronite S.	12. 5	12. 5	45. 6
Bioagents	-	-	-
B. subitlis.	00. 0	22. 2	47. 7
T. harzianum	00. 0	10. 0	26. 9
Control	20. 0	62. 5	72. 9
LSD 0. 05	0. 94	2. 0	1. 9

(1)- According to Tubers no. (2)- According to Tubers weight

were 52. 4, 50. 0, 42. 0, 11. 6 and 7. 5 %, respectively. Results showed that the most reduction of enzyme activity obtained with Starner (89. 8% inhibition) followed by streptomycin sulfate (87. 8 % inhibition), Micronite Soreil (78. 2 % inhibition), *T. harzianum* (62. 6 %inhibition) and *B. subtilis* (46. 3 % inhibition), respectively, at second concentration (Table, 2). The values of relative loss in viscosity were 1. 5, 1. 8, 3. 2, 5. 5 and 7. 9 %, respectively.

Starner gave the best inhibition to secrete of Cx enzyme yield, followed by streptomycin sulfate, *T. harzianum, B. subtilis* and Micronite Soreil at the first concentration after 72h of incubation (Table, 2). The relative loss in viscosity values were 9. 2, 9. 3, 13. 2, 13. 6 and 16. 4 %, while the Cx enzyme reduction were 48. 9, 48. 3, 26. 7, 24. 4 and 8. 9 %, respectively. At the second concentration, streptomycin sulfate, Starner, Micronite Soreil, *B. subtilis* and *T. harzianum* reduced the Cx enzyme activity, where the relative loss in viscosity were 3. 8, 4. 1, 5. 4, 11. 1 and 12. 0 %, respectively. The values of Cx enzyme activity reduction were 78. 9, 77. 2, 70. 0, 38. 3 and

33. 3 %, respectively (Table, 2).

Our results suggested that the Starner, *B. subtilis* and *T. harzianum* were strangely effective in reducing the PG enzyme yield comparing with the control as well as streptomycin sulfate and Micronite Soreil treatment. The PME enzyme activity was more sensitive to Starner and *T. harzianum* comparing with *B. subtilis*, Micronite Soreil and streptomycin sulfate. Results indicated that the Cx activity reduced by Starner, and streptomycin sulfate comparing with Micornite Soreil, *T. harzianum* and *B. subtilis*. Finally, It is clear that the Starner, *B. subtilis* and *T. harzianum* reduced the pectolytic enzymes(PG and PME), while Starner and streptomycin sulfate reduced the cellulolytic enzyme (Cx). It is revealed that these material can be play an important role in controlling the bacterial soft rot disease, especially the number of compounds which used as a bactericide is very limited[6, 28].

Population of *E. Carotovora* Subsp. *Carotovora*: The streptomycin sulfate, Starner, Micronite Soreil. *B. subtilis* and *T. harzianum* at the tested concentrations were powerful bactericide effect *in vitro* tests (Table, 3). Population of *E. carotovora* subsp. *carotovora* were reduced in treated culture medium after 24, 48, and 72 h of incubation periods, comparing with the control. The values of bacterial count reduction were ranged from 7. 2 to 94. 0 %, from 4. 8 to 60. 4 % and from 8. 2 to 87. 2 % after incubation at 24, 48 and 72 h, respectively, comparing the control. The strongest bactericide effect obtained with *B. subtilis*, where the bacterial count reduction ranged from 76. 7 to 94. 0%, followed *T. harzianum* (Reduction from 30. 1 to 91. 6 %), Streptomycin sulfate (Reduction from 36. 2 to 90. 4 %), Starner (Reduction from 45. 3 to 84. 3 %) and Micronite Soreil (Reduction from 7. 2 to 56. 6 %), respectively. There were significant differences recorded between the inhibitory effect of treatments, between the effect of concentrations and between the incubation periods. It is obvious that the effect of tested material as bactericide was clear after 24 and 48 h of incubation, but after 72 h of incubation at 30 ^{0}C the bacterial exude may be play role in suppressive the bacterial count. Our results revealed that the tested bactericide and bioagent treatments were the more effective than the control treatment in reducing the enzymes activities and population count of bacteria[6, 7, 9].

Field Experiment:

Soft Rot Incidence:

At Harvest: The field application of streptomycin sulfate*, T. harizanum, B. subtilis* protected the daughter potato tuber in treated plants against soft rot disease, where the percentage of softening tubers were zero, comparing with untreated plants (Table 4). Data also showed that the field application of Starner and Micronaite Soreil as seed tuber dressing were less effective in protection potato tubers free from softening. The percentage of softening tubers were 11. 1, 11. 0 and 12. 5 %, respectively, comparing with control (20. 0 %). It is obvious that the treatment of seed pieces, as pre-sowing application, with streptomycin sulfate, *T. harzianum* and *B. subtilis* may significantly contribute to soft rot disease suppression during plant production[33]. Therefore. no soft rot symptoms occurred on the daughter tubers at harvest[11, 34, 35].

After Storage: After 3 months of storage, the percentage of potato decay, causing by soft rot disease, was 10. 0 % with *T. harzianum*, 12. 5 % with Micronite Soreil, 22. 2 % with *B. subtilis*, 25. 0 % with Starner and 33. 3 % with streptomycin sulfate comparing with 62. 5 % in the un-treated plants control (Table, 4). It is obvious that the *T. harzianum*, Micronite Soreil and *B. subtilis*, respectively, were the most effective in reducing the soft rot decay in stored potato tubers. This study demonstrated that stored daughter potato tubers can be stored for 12 weeks, when seed pieces were treated with bioagents and / or Micronite Soreil. The weight-loss percentage of stored potato tubers are shown in Table (4). The weight-loss percentage ranged from 26. 9 % to 75. 4 % after 3 months of storage comparing with the control. The bio-agents treatment (*T. harzianum* and *B. subtilis*) gave the best results in reducing the percentage of weight-loss of potato tubers in relation to streptomycin sulfate and / or control[36, 35, 28]. These results demonstrated that *T. harzianum, B. subtilis* and streptomycin sulfate treatments can be efficient method for disinfected potato tubers, easily field applied to produce the healthy potato tubers stored for long time[34]. Therefore, the use pr-treatment of potato tubers with chemicals and bio-agents can be prevent initial infection with soft rot disease and multiplication of soft rot pathogen.

Effect on Vegetative Growth: Data in Table (5) show that applied the bactericide and bioagent treatments as seed pieces dressing increased both lengths of stem (plant height) and number of leaves in treated plants than untreated plants. It is clear that applied

Table 5: Effect of bactericide and bioagent treatments on vegetative growth of potato plants.

Treatments	Vegetative characters			
	Stem height (cm.) / plant		Leaves no. / plant	
	Average	Increase %	Average	Increase %
Bactericides:	-	-	-	-
Streptomycin S.	66. 7	22. 8	52. 0	129. 1
Starner	63. 0	16. 0	43. 0	89. 4
Micronite S.	55. 0	01. 3	40. 7	79. 3
Bioagents.	-	-	-	-
B. subitlis.	59. 7	10. 0	37. 3	64. 5
T. harzianum.	62. 7	15. 5	43. 0	89. 4
Control.	54. 3	-	22. 7	-
LSD 0. 05	1. 9	-	1. 9	-

Table 6: Effects of bactericide and bioagent treatments on, tuber weight average and tubers yield for potato plant.

Treatments	Tuber weight (g)		Tuber yield (Kg)	
	Average	Increase %	Average	Increase %
Bactericides	-	-	-	-
Streptomycin S.	115. 2	44. 4	1. 04	62. 5
Starner	87. 7	09. 9	0. 70	10. 0
Micronite S.	91. 7	14. 9	0. 73	15. 1
Bioagents	-	-	-	-
B. subitlis.	83. 0	04. 0	0. 75	17. 1
T. harzianum	89. 0	11. 5	0. 89	39. 5
Control	79. 8	-	0. 64	-
LSD 0. 05	2. 4			

Table 7: Percentage (%) of potato tubers weight at different diameter degree

Treatments	Tubers weight % at different diameter degree			
	> 20 mm	20-40 mm	45-55mm	60-70mm
Bactericides				
Streptomycin S.	0. 0	8. 3	22. 4	69. 4
Starner	0. 0	0. 0	60. 4	39. 4
Micronite S.	0. 0	19. 0	28. 7	52. 3
Bioagents				
B. subitlis.	0. 0	22. 3	35. 5	42. 2
T. harzianum	1. 2	22. 5	27. 5	48. 9
Control	0. 0	5. 1	58. 9	35. 9

streptomycin sulfate, Starner, *T. harzianum, B. subtilis* and Micronite Soreil gave the highest value of plant height comparing with untreated plants, respectively. The higher percentage of increase of plant height, than control, were obtained with streptomycin sulfate (22. 8 %), Starner (16. 0 %), *T. harzianum* (15. 5 %) and *B. subtilis* (10. 0 %), respectively. Results of field application show that the highest value of leaves numbers was obtained than control (Table, 5). The higher percentage of increase of leaves numbers were as follow: streptomycin sulfate (129. 1 %), Starner (89. 4 %), *T. harzianum* (89. 4 %), Micronite Soreil (79. 3 %) and *B. subtilis* (64. 5 %), respectively. Results suggest that the used treatments increased the plant growth expressed as plant height and numbers of leaves. This increased may be due the treatments improved the growth of seed pieces to give vigorous plants [36].

Effect on Potato Tubers Yield: Potato tubers yield per plant was trend with the same observation of percentage the softening tubers (Table, 6). The higher increase in potato yield per plant was obtained with streptomycin sulfate (62. 5 %), *T. harizanum* (39. 5 %) and *B. subtilis* (17. 1 %), respectively. The moderate increase in potato tuber yield with obtained with Micronite Soreil (15. 1 %) and Starner (10. 0 %), respectively. Data in Table (6) show that the tested bactericides and bioagents gave the variance reaction with the average of tuber weight. Streptomycin sulfate treatment produce the best average weight of tuber, followed by Micronite Soreil, *T. harzianum*, Starner and *B. subtiils*, respectively. Results suggest that the number, average of tuber weight and tubers yield per plant increased when seed pieces of potato tubers were treated with the tested bactericides and bioagents [14]. It is concluded that the careful pre-sowing application of some bactericides and bio-agents gave the best results in increasing the tuber yield per plant and average of potato tuber weight[37].

The diameter of harvested potato tubers under different field applications of bactericides and bioagents was ranged from > 20 mm. to 70 mm (Table, 7). It is clear that the application of the most tested treatments gave the highest amounts of potato tubers at diameter from 60 to 75 mm. Details of the percentage of tubers weight resulting from treatments at different diameter is shown in Table (7). The bactericides and bioagent treatment not only significantly reduced the disease severity but also yield in naturally infested fields.

Results showed that the disease incidence of soft rot can be reduced in the field and in storage by treatments of seed pieces pre-planting with bio-agents and some tested bactericides. Among the tested treatments streptomycin sulfate, *T. harzianum* and *B. subtilis* could be increase the plant yield of tubers and reduction the incidence of soft rot disease in both the field and the storage. Results suggested that the treatments were sufficiently effective against soft rot disease in field or in storage, and increased potato quality and yield[38].

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