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NATURAL EXTRACTS OF MUSHROOM AND GARLIC AS BACTERICIDE ALTERNATIVES AGAINST POTATO SOFT-ROT BACTERIA, *Erwinia carotovora* subsp. *carotovora*.

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ABSTRACT

Current study aimed to search for safe and effective natural products to control the potato soft-rot disease, *Erwinia carotovora* subsp. *carotovora*. Antibacterial efficiency of edible mushroom extracts (MEs) and garlic essential oil (EO) were compared with two bactericides; streptrol and oxolinic acid as well as two fungicides; mancopper and copper oxychloride. Bioassay experiments were conducted using the paper disc diffusion and potato slices methods. Results showed that both natural extracts and synthetic pesticides exhibited marked *in vitro* antibacterial activity particularly streptrol and the chloroform extract of the mushroom which showed 3.4-fold inhibitory effect compared with garlic EO. Also, the chloroform extract of mushroom showed similar activity to mancopper and copper oxychloride. Mixing garlic oil (1 mg/ml) with streptrol or oxolinic acid (at 0.05 mg/ml) synergized the inhibitory effect of both synthetic bactericides. The overall results suggest the potential use of the edible mushroom extracts and garlic essential oil for the control of soft-rot disease on potatoes.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important vegetable crop in Egypt for both local consumption and exportation. Potato bacterial soft-rot disease caused by *Erwinia carotovora* subsp *carotovora* is a very destructive disease to potato tubers during the plant growth in the field and in storage. One of the most important methods to control the bacterial soft rot disease in potato tubers, all over the world, was the use of chemical bactericides and fungicides (**Van der Zwet and Kail, 1979; Saleh and Huang, 1997**). The deleterious effects of synthetic pesticides to consumers' health, environment, and the development of resistance by pathogens are major problems. Therefore, there is a demand to develop new safe and fast biodegradable natural materials that have maximum efficacy against the pathogen, minimal detrimental environmental side effects, and non-dangerous to the consumers (**Singh, 1994; Mason and Mathew, 1996**).

Natural products are important sources of new agrochemicals to control plant diseases (**Cardellina, 1988**). Ten furostanol (voghieroside A1/A2 and voghieroside B1/B2, voghieroside C1/C2, and voghieroside D1/D2 and E1/E2) and spirostanol (agigenin 3-O-trisaccharide and gitogenin 3-O-tetrasaccharide) saponins were identified in the polar extract of garlic bulbs *Allium sativum* L., var. Voghiera (**Lanzotti *et al*., 2012**). Those saponins showed antifungal activity against *Botrytis cinerea* and *Trichoderma harzianum* (**Lanzotti *et al*., 2012**). In addition, aqueous and methanol extracts of bulb extracts showed antimicrobial activity **(Meriga *et al*., 2012)**. Aqueous extract exhibited antibacterial activity against *Bacillus subtilis, Staphylococcus aureus Escherichia coli*, and *Klebsiella pneumonia* strains and antifungal activity against *Candida albicans* while the methanol extract had antimicrobial activity against all the tested microorganisms except for *Staphylococcus aureus* and *Candida albicans* **(Meriga *et al*., 2012)**.

Aqueous-methanolic extracts of wild mushroom, *Russula delica* and *Fistulina hepatica* extracts had antibacterial effects against

Escherichia coli, *Morganella morganni*, *Pasteurella multocida*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Streptococcus agalactiae*, and *Streptococcus pyogenes* at concentrations range between 10 to 20 mg/ml **(Alves *et al.*, 2012)**. Strobilurin A was isolated from the wild mushroom, *Strobilurus tenacellus* and had been derivativezed to the widely used broad-spectrum fungicidally pyrimidinyl dioxy strobilurins (**Lamberth, 2012**). Current study aimed to find bactericide alternatives through the extraction and examination of the bactericidal and synergistic activity of mushroom extractives and garlic volatile oil against soft-rot bacteria, *Erwinia carotovora* subsp *carotovora* isolate.

MATERIAL AND METHODS

Synthetic Compounds and Natural Extracts

Streptrol WP 21.3% (steptomycin sulfate), starner WP 20% (oxolinic acid), mancopper, and copper oxychloride were bought from local companies. Chloroform, diethyl ether, ethanol, petroleum-ether solvents of analytical grade were purchased from local suppliers.

Preparation of Natural Extracts of Mushroom (ME)

Cap and stem of field (*Agaricus bisporus*) and oyster mushroom (*Pleurotus osteatus*) were air dried at room temperature for 2 wk and then in the oven at a temperature < 50° C for 3 d. Fungi samples were ground using an electric blender to a fine powder. Mushroom samples were extracted successively in Soxhlet apparatus with chloroform, petroleum-ether (60 - 80°C), diethyl ether, and ethanol (95%). Before each successive extraction, the powder was carefully spread on sheet of paper to dry at room temperature. After extraction with petroleum-ether, the powder was alkalinized with dilute ammonium hydroxide before it was extracted with diethyl ether and ethanol. Extracts were dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure in rotary evaporator (Unipan vacuum rotary evaporator type 350P, Poland). Dried crude extracts were kept in tightly closed brown bottles and stored in -20° C until use.

Extraction of Volatile Oil of Garlic

Volatile oil content of garlic was isolated from the macerated cloves of garlic, *Allium sativum* Linn. Batches of garlic gloves were macerated and reconstituted by soaking overnight in distilled water and then thoroughly mixed in blender. The mixture was steam-distilled using steam distillation apparatus connected with oil Clevenger trap (**Guenther, 1952**). The oily layer was separated and shaken with excess of anhydrous diethyl ether in a separator funnel. The ether layer was dried with anhydrous sodium sulfate. The solvent was completely removed under vacuum using a rotary evaporator. The resulting oil was stored in a dark bottle at -20° C until use.

Tested Bacteria and Inoculum Preparation

Erwinia carotovora subsp. *carotovora* was obtained from Faculty of Agriculture, Ain Shams University, Egypt. Bacterial Inoculum was prepared from a 24 h-old culture. Bacterial suspension was prepared by scraping the bacterial growth of one Petri dish (9 cm diameter) in 10 ml sterile water and used for the pathogenicity tests.

Inoculation of Potato Tubers

Potato tubers were surface sterilized using 95% ethanol for 1 min and then flamed. Tubers were cut into 1 cm slices and each slice was placed in a sterile Petri dish and lined with moistened absorbent paper. Tuber slices were inoculated with 100 µl of the bacterial suspension at the center of each slice. Control tuber slices were treated with 100 µl sterilized water. Dishes were incubated at 28±2°C for 72 h. Soft-rot symptoms were recorded following the scale method described by **Hide and Cayley (1982)** and **Bartz (1999)**. Results were scored as the following: + = arrested rot < 1 cm, ++ = small active rot < 2 cm, and +++ = large active rot > 2 cm.

***In Vitro* Antibacterial Activity Test**

The antibacterial activity of the tested compounds (natural extracts and pesticides) was carried out following the paper disc plate method (**Loo *et al.*, 1945; Thornberry, 1950**). Tested compounds were prepared

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as the following: streptrol and oxolinic acid (bactericides) (8 concentrations from 0.005 to 0.64 mg/ml); mancopper and copper oxychloride (fungicides) (7 concentrations from 0.1 to 6.4 mg/ml); mushroom extracts (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mg/ml), and garlic oil (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 mg/ml). Controls were treated with distilled water. Petri dishes with a medium of nutrient dextrose agar were inoculated by spreading 0.1 ml of the 24 h-old bacterial suspension of broth culture using L-shaped spatula. Discs were impregnated in different concentrations of each of tested compounds and placed onto the surface of inoculated plates. Plates were incubated at 28±2°C for 48 h. Three replicate of each treatment (discs) were used. Check filter paper discs were placed on the surface of inoculated plates after impregnating within sterile water as control. The diameter of the inhibition zone around the disc was measured and means were calculated.

Joint Inhibitory Effects of Natural Extracts and Streptrol and Oxolinic Acid against *E. c. carotovora*

Approx. 0.05 mg/ml of the bactericides (streptrol or oxolinic acid) were mixed with 0.1 mg/ml of each of the mushroom extracts (field and oyster) or with 1.0 mg/ml of garlic volatile oil. The different combinations were tested against the bacteria. Check filter paper discs impregnating in sterilized water were used as control. Inhibition zones were measured after incubating the bacterial culture at 28±2°C for 48 h.

***In Vivo* Inoculation of Potato Tuber Slices**

Healthy potato tubers were surface-sterilized with 95% ethanol for 1 min and flamed and then were cut into 1 cm slices. Three potato slices were dipped in each of the concentrations of the used bactericides, fungicides, and natural products for 30 sec. After that, artificially infected slices were made by depositing of 0.1 ml of the bacterial suspension onto the center of each tuber disc slice. The potato slices were incubated at 28+2°C for 72 h. Soft rot symptoms were recorded according to the scale described by **Hide and Cayley (1982)** and **Bartz (1999)** as the following : - = no rotting, + = arrested rot < 1 cm, ++ = small active rot < 2 cm, and +++ = large active rot > 2 cm.

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Statistical Analysis

Data were statistically analyzed as completely randomized design (CRD). Data were tested using the general linear model (GLM) procedure of the statistical analysis system (SAS) (version 9) and means were compared using the least significant difference (LSD) at $P<0.05$.

RESULTS

***In Vitro* Antibacterial Activity of Mushroom Extracts and Garlic Oil**

It is evident from the data presented in Table 1 that different mushroom extracts caused significant inhibitory effect to the growth of the bacteria compared with non-treated control. In addition, the chloroform extract of mushroom was the most suppressive to the bacteria with an average inhibition zone ranged from 1.83 and 3.50 cm and from 1.27 to 3.07 for field and oyster mushroom, respectively and followed by the ether extract. The extracts of field mushroom were more potent than extracts of the oyster mushroom.

Garlic oil at concentrations ranged from 4.0 to 7.0 mg/ml were tested against soft rot bacterial isolates. All of the tested concentrations of garlic oil had inhibitory effect to *E. c. carotovera* isolates (Table 1 and Figure 1). The results revealed that by increasing the garlic oil concentrations, the inhibition zone was increased.

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Table 1. Antibacterial activity as an average inhibition zone (AIZ) in cm of extracts of field mushroom (FM), oyster mushroom (OM), and garlic oil against soft rot bacteria *E. c. carotovora* after 48 h of incubation.

Mushroom Extracts									Garlic Oil	
Conc. (mg/ml)	Petroleum ether		Ether		Ethanol		Chloroform		Conc. (mg/ml)	AIZ
	FM	OM	FM	OM	FM	OM	FM	OM		
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00
0.5	1.23	1.03	1.43	1.20	1.07	0.97	1.83	1.27	4.0	1.03
1.0	1.70	1.13	1.83	1.40	1.17	1.07	1.90	1.57	4.5	1.20
1.5	1.93	1.37	2.13	1.77	1.30	1.27	2.30	1.97	5.0	1.40
2.0	2.17	1.43	2.43	2.07	1.33	1.30	2.57	2.30	5.5	1.57
2.5	2.23	1.67	2.63	2.20	1.37	1.47	2.77	2.50	6.0	1.87
3.0	2.43	1.80	2.80	2.30	1.43	1.57	3.07	2.77	6.5	2.03
3.5	2.77	2.17	3.03	2.50	1.67	1.73	3.37	2.90	7.0	2.17
4.0	3.03	2.40	3.13	2.63	1.93	2.03	3.50	3.07	-	-
$LSD_{0.05}$	0.05	0.06	0.06	0.02	0.08	0.06	0.08	0.07		0.08

Data were average of three replicates.

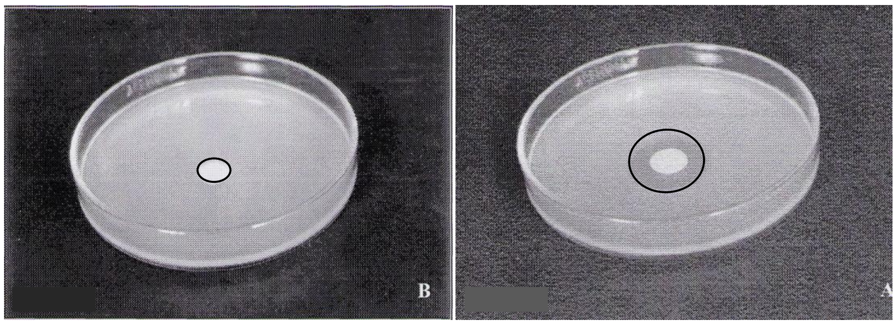

Figure 1. Sensitivity of soft rot bacteria, *E. c. carotovora* to garlic oil; (A) garlic essential oil and (B) control.

Antibacterial Activity of Streptrol, Oxolinic Acid, Mancopper and Copper Oxychloride

Sensitivity of soft rot bacteria to the tested bactericides (streptrol and oxolinic acid) was shown in Table 2. Results indicated that streptrol and oxolinic acid were effective bactericides in a concentration-dependent manner with concentrations ranged from 0.005 to 0.64 mg/ml. Streptrol tended to be a strong inhibitor of the bacterial growth with an average inhibition zone that ranged from 2.33 to 6.00 cm compared to oxolinic acid (2.17 - 5.73 cm).

Data presented in Table 2 and Figures 2 and 3 revealed that t the fungicides mancopper and copper oxychloride caused different degrees of growth inhibition of *E. c. carotovora*. The average inhibition zone ranged from 2.1 to 5.3 and 1.6 to 4.7 cm for mancopper and copper oxychloride, respectively. In addition, the data indicated that increasing concentration of each fungicide from 0.1 to 6.4 mg/ml had increased the inhibition zone of bacterial growth in a concentration dependent manner.

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Table 2. Antibacterial activity of streptrol, oxolinic acid, mancopper, and copper oxychloride against *E. c. carotovora* after 48 h of treatment, shown as an average inhibition zone in cm.

Average of inhibition zone (cm)*					
Bactericides			Fungicides		
Conc (mg/ml)	**Streptrol**	**Oxolinic acid**	**Conc (mg/ml)**	**Mancopper**	**Copper oxychloride**
0.00	0.00	0.00	0.00	0.00	0.00
0.005	2.33	2.17	0.1	2.1	1.6
0.01	2.50	2.40	0.2	2.4	1.9
0.02	2.83	2.87	0.4	2.7	2.4
0.04	3.20	3.17	0.8	3.2	2.9
0.08	4.07	3.83	1.6	3.9	3.4
0.16	5.10	4.63	3.2	4.4	4.1
0.32	5.40	5.10	6.4	5.3	4.7
0.64	6.00	5.73			
$LSD_{0.05}$	1.13	0.06		0.06	0.09

*Data are means of three replicates

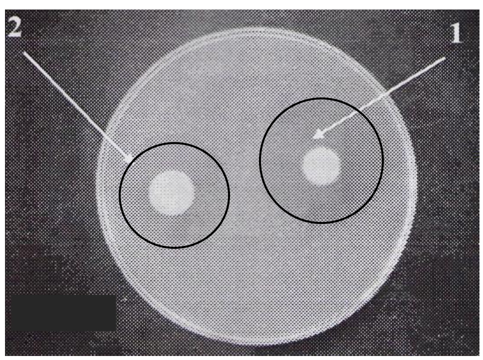

Figure 2. Sensitivity of soft rot bacteria, *E. c. carotovora* to tested bactericides: 1; streptrol and 2; oxolinic acid.

Joint Inhibitory Effect of Mushroom Extracts and Garlic Oil against *E. c. carotovora*

Data in Table 3 showed the sensitivity of soft rot bacteria to streptrol or oxolinic acid at 0.5 mg/ml mixed with mushroom extracts at 0.1 mg/ml concentration. Results indicated that mushroom extracts antagonized (decreased) the inhibitory effect of both streptrol or oxolinic acid on soft rot bacterial isolate, as shown in Table 3.

Table 3. Joint effect of mushroom extracts (ME) and bactericides; streptrol and oxolinic acid against *E. c. carotovora*.

Treatment[a]	Control	Average of Inhibition Zone				
		Streptrol or Oxolinic Acid, 0.05 (mg/ml)	OM[b] (0.1 mg/ml)	FM[c] (0.1 mg/ml)	Streptrol or Oxolinic acid : ME (2:1)	
					OM	FM
1	0.00	3.30	0.27	0.30	3.27	3.37
2	0.00	3.30	0.83	0.97	3.93	4.00
3	0.00	3.30	0.30	0.27	3.33	3.37
4	0.00	3.30	0.00	0.00	2.97	3.10
5	0.00	3.30	1.00	1.07	4.03	4.13
6	0.00	3.27	0.27	0.30	3.03	3.27
7	0.00	3.27	0.83	0.97	3.87	4.00
8	0.00	3.27	0.30	0.27	3.07	3.27
9	0.00	3.27	0.00	0.00	2.83	3.03
10	0.00	3.27	1.00	1.07	4.03	4.06
$LSD_{0.05}$		NS	0.53	0.55	0.17	0.21

[a]Treatments were 1: Streptrol + petroleum ether extract of mushroom, 2: Streptrol + ether extract of mushroom, 3: Streptrol + chloroform extract of mushroom, 4: Streptrol + ethanol extract of mushroom, 5: Streptrol + chloroform crude, 6: Oxolinic acid + petroleum ether extract of mushroom, 7: Oxolinic acid + ether extract of mushroom, 8: Oxolinic acid + chloroform extract of mushroom, 9: Oxolinic acid + ethanol extract of mushroom, and 10: Oxolinic acid + chloroform crude, [b]OM: oyster mushroom, and [c]FM: field mushroom. Means were compared using the Least Significant Difference *post-hoc* Test ($P \leq 0.05$).

Streptrol and oxolinic acid individually had more inhibitory effect at 0.5 mg/ml to the growth of *E. c. carotovora* isolate, with average inhibition zones of 3.3 and 3.27 cm, respectively, compared to their mixtures with mushroom extracts.

Results in Table 4 showed that garlic oil at a concentration of 1.0 mg/ml tended to increase the inhibitory effect of bactericides, streptrol and oxolinic acid. The garlic oil/ streptrol mixture was effective inhibitor of the bacterial isolate with average inhibition zone of 3.33 cm compared to the inhibition zone of streptrol itself (3.30 cm).

Table 4. Joint inhibitory effect of garlic oil extract and bactericide streptrol or oxolinic acid on *E. c. carotovora*.

Treatment	Average Inhibition Zone (cm)	
	Control	Garlic oil (1.0 mg/ml)
Garlic (1 mg/ml)	0.00	0.00
Streptrol (0.05 mg/ml)	0.00	3.30
Oxolinic acid (0.05 mg/ml)	0.00	3.13
$LSD_{0.05}$		0.16

In Vivo Inhibitory Effects of Inoculated Potato Tuber Slices

Data of inhibitory effect of streptrol, oxolinic acid, mancopper, copper oxychloride, mushroom extracts and garlic oil on *E. c. carotovora* isolate were shown in Tables 5. The immersion in a 0.002 mg/ml solution of streptrol and oxolinic acid for 30 s prevented the development of soft rot bacteria on inoculated potato tuber slices.

Results in Table 5 show that soft rot did not develop on potato slices when mancopper was applied at a concentration of 0.4 mg/ml, while it required a higher dose of copper oxychloride (1.6 mg/ml) to suppress the soft rot symptoms. Similarly, the immersion of potato slices in the chloroform extract of mushroom (2.0 mg/ml) has prevented the development of soft rot symptoms of *E. c. carotovora*.

Table 5. Sensitivity of soft rot bacteria *E. c. carotovora* to bactericides; streptrol and oxolinic acid and fungicides; mancopper and copper oxychloride using potato tuber slice test, measured as the rotting rate of potato slices.

Bactericides			Fungicides		
Conc. (mg/ml)	**Streptrol**	**Oxolinic acid**	**Conc. (mg/ml)**	**Mancopper**	**Copper oxychloride**
0.00	+++	+++	0.0	+++	+++
0.005	+	+	0.1	++	++
0.01	+	+	0.2	+	++
0.02	-	-	0.4	-	++
0.04	-	-	0.8	-	+
0.08	-	-	1.6	-	-
0.16	-	-	3.2	-	-
0.32	-	-	6.4	-	-
0.64	-	-	-	-	-

"+++"large active rot, "++"small active rot, "+"arrested rot, and "-"no rotting

On the other hand, treatment with the petroleum ether extract of mushroom at concentration of 3.0 mg/ml prevented the progression of soft rot symptoms on inoculated potato slices. Data presented in Table 6 show that chloroform extract inhibited the development of rotting of potato slices at 2 mg/ml of either field or oyster mushrooms. Also, ether and petroleum-ether extracts of field mushroom and garlic oil at 3.0 mg/ml suppressed the growth of bacterial isolate. On the other hand, the ethanol extract of field mushroom did suppress the disease development at 3.5 mg/ml but not the ethanol extract of oyster mushroom.

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Table 6. Sensitivity of soft rot bacteria *E. c. carotovora* to mushroom extracts and garlic oil using tuber slice test via the measurement of rotting rate (RR) of potato slices.

Conc. (mg/ml)	Mushroom Extracts								Garlic oil	
	Petroleum ether		Ether		Ethanol		Chloroform			
	FM*	OM	FM	OM	FM	OM	FM	OM	Conc. (mg/ml)	RR
0.0	+++	+++	+++	+++	+++	+++	+++	+++	0.0	+++
0.5	++	++	++	++	++	++	++	++	4.0	++
1.0	+	+	+	+	++	++	+	+	4.5	++
1.5	+	+	+	+	++	++	+	+	5.0	+
2.0	+	+	+	+	+	+	-	-	5.5	+
2.5	+	+	+	+	+	+	-	-	6.0	+
3.0	-	-	-	+	+	+	-	-	6.5	-
3.5	-	-	-	-	-	+	-	-	7.0	-

*Mushroom (FM) and oyster mushroom (OM), "+++" large active rot, "++" small active rot, "+" arrested rot, and "-" no rotting.

DISCUSSION

Erwenia carotovora subsp *carotovora* is the causal agent of the soft rot disease of potato tuber in storage and the field where early decay of mother tubers or seed tuber pieces occur (**Perombelon and Kelman, 1980**; **Zhijian *et al.*, 2000**). It is very important to determine the effectiveness of natural compounds at different concentrations to detect the suitable concentration for combating *E. c. carotovora* concurrently with synthetic bactericides. In current study, pathogenicity tests of *E. c. carotovora* were carried out on potato tuber slices. On inoculated potato tuber slices, the bacteria caused severe soft rot symptoms. These results were in agreement with those of several authors who pointed out that *E. c. carotovora* caused soft rot in potato tubers and other crops (**Smith and Bartz, 1990; Mickalik *et al*., 1992; Saleh and Huang, 1997; Helias, 2000**).

In vitro screening of tested compounds showed that streptrol (streptomycin sulfate) and oxolinic acid (starner S–0208) had inhibitory effect to soft rot bacterial at concentrations ranged from 0.002 to 6.4 mg/ml, in a concentration dependent manner. These findings were supported by many authors who found that streptomycin sulphate was more effective in controlling *E. amylovora* pathogen than copper fungicides (**Seif El-Nasr and Ali, 1989; Tsiantos and Psallidas, 1990**). Sumitomo-0208 compound (oxolinic acid analogue) controlled *E. amylovora* (**Barakat *et al.*, 2002**) and was effective against *E. c. carotovora* isolates (**Abd El-Khair, 2004**).

Also, the present results revealed that mancopper was more effective against soft rot isolate at 0.02 mg/ml or higher, in a concentration dependent manner, than copper oxychloride. **Haggag (1995)** found that the growth of *E. c. carotovora* was inhibited by mancopper, mancozan and copper oxychloride. Also, **Barakat et al. (2002)** reported that copper oxychloride and copper sulphate had a low effect on *E. amylovora*, while cuprozan and mancopper were more effective.

Mushroom extracts caused a significant inhibitory effect on the growth of *E. c. carotovora*, in a concentration dependent manner, compared with the non-treated control. Chloroform extract was the most suppressive to the tested bacteria with concentrations ranged from 0.5 to 4.0 mg/ml. Also, extracts of field mushroom were more potent inhibitors than that of the oyster mushroom. Three antibacterial substances were extracted from mushroom, *Lentinus edodes* by chloroform, ethyl acetate, and water and these extracts exhibited good activity against *Strptococcus* sp., *Actinomyces* sp., *Lactobacillus* sp., *Prevotella* sp. and *Prophyromonas* sp. (**Hirasawa *et al.*, 1999**). Also, **Lamberth (2012)** reported the development of strobilurin A compound from the wild mashroom, *Strobilurus tenacellus* and it had been widely used as a broad-spectrum fungicide as pyrimidinyldioxy strobilurins.

Garlic oil caused significant inhibitory effect to *E. c. carotovora* growth with concentrations ranged from 4.0 to 7.0 mg/ml. These results were in agreement with those obtained by **Lis-Balchin *et al*. (1998), Aysan *et al.* (2000), Qiao *et al.* (2001) and Benkebila (2004)** on the antibacterial activity of garlic oil. Furostanol and spirostanol saponins were identified in the polar extract of garlic bulbs *Allium sativum* L., var. Voghiera, and showed antifungal activity against *Botrytis cinerea* and *Trichoderma harzianum* (**Lanzotti *et al*., 2012**).

Effective bactericides were tested in binary mixtures with natural extracts of mushroom and garlic oil to minimize the dose of synthetic chemicals and maximize the effectiveness of natural extracts. It was obvious that mixing of the synthetic bactericides; streptrol or oxolinic acid with mushroom extracts has decreased (antagonized) the bactericidal effect of streptrol or oxolinic acid on *E. c. carotovora*. On contrary, garlic oil increased (synergized) the bactericidal effect of streptrol or oxolinic acid. This finding is of great economic and environmental importance.

Results of the *in vivo* studies revealed that streptrol, oxolinic acid, mancopper and copper oxychloride at concentrations of 0.002, 0.002, 0.002, and 0.08 mg/ml, respectively, prevented the development of soft rot on inoculated potato tuber slices. On the other hand, chloroform extract of mushroom at 2 mg/ml suppressed the growth of soft rot symptoms on inoculated potato slices, while the petroleum ether and ether extracts inhibited the development of soft rot at 3 mg/ml of each extract. Also, garlic oil at 6.5 mg/ml was growth inhibitor of *E. c. carotovora*. Thus, the overall results suggest the potential use of the edible mushroom extractives and garlic oil as bactericide alternatives in the control of soft rot disease on potatoes.

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الملخص العربي

مستخلصات فطر عيش الغراب ونبات الثوم كبدائل مبيدات لمكافحة بكتريا العفن الطري (*Erwinia carotovora* subsp *carotovora*) في البطاطس

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هدفت الدراسة الحالية الى البحث عن بدائل للمبيدات البكتيرية من خلال دراسة التأثير الإبادى لبعض المستخلصات الطبيعية لعيش الغراب والثوم ضد بكتريا العفن الطري (*Erwinia carotovora* subsp. *carotovora*) في نباتات البطاطس. تم مقارنة التأثير الفعال لمستخلصات عيش الغراب والثوم وبعض المبيدات الفطرية (mancopper and copper oxychloride) والبكتيرية (streptrol and oxolinic acid) ضد بكتريا العفن الطري باستخدام طريقتي الانتشار من خلال أقراص ورقية وشرائح درنات البطاطس (paper disc diffusion and potato slices). وقد أظهرت النتائج أن المستخلصات الطبيعية والمبيدات أعطت نتائج جيدة في مكافحة بكتريا العفن الطري حيث ان مستخلص الكلوروفورم من عيش الغراب قد أعطى تأثير ابادى يفوق تأثير زيت الثوم بـ 3.4 ضعف. مستخلص عيش الغراب باستخدام مذيب الكلوروفورم أعطى نتائج إبادة مثل التي أعطتها مبيدات الـ mancopper and copper oxychloride. كما أن تجارب خلط زيت الثوم (بتركيز 1 مجم/مل) مع مبيدى الـ streptrol or oxolinic acid (بتركيز 0.05 مجم/مل من كل منهما) قد أدى الى تحسين وزيادة الكفاءة الابادية لتلك المبيدات. النتائج التي يوضحها البحث الحالي تبين الدور الهام الذي يمكن أن تحدثه مستخلصات عيش الغراب والزيت الطيار للثوم كبدائل أو منشطات لفعل المبيدات البكتيرية ضد مسبب مرض العفن الطري في نباتات البطاطس.