EVALUATION OF SOME FUNGICIDES EFFECTIVENESS IN CONTROL OF BLACKLEG AND COMMON SCAB OF POTATO

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Abstract

Antimicrobial potential of Controller, Impose, Phostrol, Ridomil Gold $_{plus}$, and Roxyl Plus was determined for *Pectobacterium atrosepticum* and *Streptomyces scabies.* The cidal effect against *P. atrosepticum* was only shown by the fungicide Controller. *S. scabies* was highly affected by Controller, Impose and Roxyl Plus compared to RidomilGold $_{plus}$, and Phostrol that did not inhibit the growth. The MIC of Controller was found to be 4000 ppm for *P. atrosepticum* and *S. scabies*. On the other hand, the MICs of Impose and Roxyl Plus were found to be 4000 ppm and 3000 ppm respectively for *S. scabies*. Application of Controller against blackleg significantly decreased the incidence of the disease in potted potato plants. Application of Controller, Impose and Roxyl Plus at 3.0g/l to control common scab significantly decreased the disease. Conclusive results were obtained by the 4.0 g/l application. Residues of ingredients of these fungicides in potato tuber 60 day after application were below the approved maximum residue level (MRL).

Keywords: Fungicides, Copper, Dimethomorph, Cymoxanil, Metiram, Phosphorus acids salts, Mefenoxam, Chemical control, Blackleg, Common scab, Potato, Residues.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered the fourth most important consumers crop after rice, wheat and maize. Potato is grown in more than 160 countries and mean per capita global consumption is 33 kg / year. The potato is consumed fresh or processed as well as many industries and food products are based on potato crop. Many pathogenic bacteria attack potato plants and are responsible for serious diseases. Among the most widely known pathogens that attack potato are being *Pectobacterium* spp. (Soft rots and blackleg) and Streptomyces sp. (Potato scabs). Blackleg affects stems and soft rot affects tubers and caused by polyphyletic pathogens (*Pectobacterium* and *Dickeya*). These Polyphyletic pathogens may be isolated from one sample. *Pectobacterium atrosepticum* is the main causal agent of blackleg disease in field in most cases. The crop losses due to these polyphyletic bacteria are varying from one country to another (Raoul des Essarts *et al.*, 2016). Potato scab is caused by different *Streptomyces* species. Common scab is a disease with worldwide occurrence and with marketing impacts that affect tuber quality due to superficial and pitted lesions that swipe potato surface. Common scab is rated among the top five diseases of potatoes, and *Streptomyces scabies* is considered the most common agent of disease. The two mentioned diseases (blackleg and common scab) present a major problem for potato growers in Egypt, especially in the last few years.

Chemical control strategies used against bacterial diseases are theoretically based on temporal eradication of the pathogen and/or by making environmental conditions unfavorable for disease development. Disease control becomes limited once the disease is initiated due to the rapid multiplication and rapid spread of bacteria, and the inability of chemicals to penetrate internal tissues (Czajkowski *et al.*, 2011).

According to earlier studies, different forms of copper compounds were the most common used fungicides. The antimicrobial efficiency of copper compounds depend more on the ability of copper compounds to retain on plant surface rather than their concentration or number of applications. The biostatic effect of cooper hinders the bacterial growth on the treated surface. Copper causes certain changes in protein structures for fungi and bacteria which lead to interrupting of protein function; and this effect is aggressive especially in moist media (Rusjan, 2012). Cymoxanil fungicide had a potential in late blight control, along with other Oomycetes tuber pathogens and black scurf in potato. The primary mode of action of cymoxanil is still unclear but it has a secondary effect on synthesis of DNA and RNA (Ziogas and Davidse, 1987). Phosphorous acid (H_3PO_3) products as Aliette and Phostrol work as fungicides by interrupting the metabolic processes and were used worldwide to control downy mildew of grapevines and vegetable crops (Magarey *et al.*, 1990 and Keinath, 2010). Metalaxyl is a systemic fungicide specifically inhibits RNA synthesis of some species of fungi. Metalaxyl is used to control disease caused by air- and soil-borne fungi of Peronosporales, which is being used extensively in Egypt as foliar spray or soil drench for controlling downy mildew and damping off (Malhat, 2012). Dimethomorph is a systemic fungicide which is used against fungi belonging to *Peronosporaceae* and genus *Phytophthora* but not *Pythium*. Sterol biosynthesis inhibits in fungal cells by Demethylation-inhibiting (DMI) fungicides (Yang *et al.* 2011). Metiram is a non-systemic fungicide of dithiocarbamate group used on ornamental crops to prevent crop damage in the field, stores and during transport (Sakr and Shalaby, 2012). Ethylenebisdithiocarbamates fungicides (mancozeb, maneb, and metiram) have multi-site mode of action, these fungicides break down to cyanide which reacts with thiol compounds in the cell and interferes with sulfhydryl groups (Holm *et al.*, 2003). Mefenoxam (recent isomer of metalaxyl) is fungicide used for managing diseases caused by *Phytophthora* spp. on ornamentals. It has selective inhibition of ribosomal RNA synthesis (Hu *et al*., 2008).

There are many compounds such as antibiotics, inorganic and organic salts separately or in combinations were tested to decrease infection by *Pectobacterium* spp. and *Dickeya* spp. to potato tubers. Antibiotics treatments before planting have led to a promise decrease in incidence of blackleg but the intensive use is being hazardous because of induced resistance in certain bacterial pathogens of humans or animals. The presence of competing bacteria in lenticels, wounds and vessels provide a good protection for them. Applications of gaseous bactericides (chlorine), which may be more successful, are impeding by the fact that their penetration into tubers is weak and can be toxic to plants (Czajkowski *et al*., 2011).

Cultivation of disease –free tubers and soil treatments would reduce infection caused by the soil inhabiting pathogen. The two main strategies to control potato common scab are freedom of tuber sets from the scab and the use of chemicals. A wide range of chemicals such as mercuric compounds, hot formaldehyde, copper sulphate, boric acid, thiophanate-methyl, mancozeb, captan, metiram, chlorothalonil, fluazinam, flusulphamide, fenpiclonil and Antibiotics to control scab disease were historically evaluated. Mercuric salts, copper salts and boric acid have proven effective as seed tuber treatments but are not acceptable for many reasons. Mancozeb, fluazinam, flusulphamide and fenpiclonil have shown promise as seed tuber treatments. Soil drenching treatments, however, as Formaldehyde, urea, formaldehyde, manganese sulphate and pentachlorobenzene have generally proven unsatisfactory. Flusulfamide dip treatment of tubers is recommended in South Africa together with chlorpicrin and quintozene for soil treatment. The uses of growth regulators or retardants as 3, 5-dichlorophenoxyacetic acid have shown some control to common scab but their effect on the crop have been unacceptable. Selection of resistant varieties can reduce common scab incidence more than any chemical treatments (Stead and Wale, 2004).

In the present work the antibacterial activity of some fungicides against *P. atrosepticum* and *S. scabies in vitro* was studied. The minimum inhibitory concentrations (MIC) of the fungicides against the pathogens in concern and the possibility of using such fungicides in the control of blackleg and common scab diseases were tried as soil drench of pots.

MATERIALS AND METHODS

Fungicides and pathogenic bacteria:

The Five used fungicides, Controller, Impose, Phostrol, RidomilGold $_{plus}$ and Roxyl Plus are shown in table (1). The pathogenic isolates of *Pectobacterium atrosepticum* and *Streptomyces scabies* were obtained from the collection of Bacterial Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. The pathogenicity test was re-performed with *P. atrosepticum* and *S. scabies* to confirm their pathogenic ability.

Table 1. Sources, active ingredients, and chemical names of different fungicides used against *P. atrosepticum* and *S. scabies*

Fungicide	Active ingredients	Chemical name	manufacture company	Local company
Controller 77.2 % WP	Dimethomorph 6.0 % w/w + Copper oxychloride 67.0 % w/w + Cymoxanil 4.2 % w/w	(*E,Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]morpholine + copper chloride oxide hydrate + 2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	Chemtura Pharma - Belgium	Aspire for Protection and Development Agricultural Project
Impose 61.8 % WG	Cymoxanil 57.0% w/v + Metiram 4.8 % w/v	2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide +zinc ammoniate ethylenebis(dithiocarbamate) - poly(ethylenethiuramdisulfide)	Agri Sciences Ltd.- Turkey	El Khaleej for Agricultural Development
Phostrol 53.6 % SL	Phosphorus acids salts 53.6 % w/v	mono-and dibasic sodium potassium and ammonium phosphate	Nufarm Americas, Inc.-USA	Cairo Chemical
RidomilGold $_{plus}$ 42.5 % WP	Mefenoxam 2.5 % w/w + Copper oxychloride 69.0 % w/w (= 40 % metallic copper)	methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-D-alaninate + copper chloride oxide hydrate	Syngenta Agro-Switzerland	Syngenta Agro-Egypt
Roxyl Plus 86.9 % WP	Metalaxyl 15 % + Copper hydroxide 53.9 % (= 35 % metallic copper)	methy N-(2,6 dimethylphenyl)-N-(methoxyacetel)-DL-alaninate + copper hydroxide (Cu (oH) $_2$)	Jiangsu Baoling Chemical Ltd.- China and Ingenieria Industrial , S.A DE C.V-Mexico	Commercial For Agricultural Supplies (CAM) and CAM for Agrochemicals

***In-vitro* effect of selected fungicides against *P. atrosepticum* and *S. scabies*:**

Controller 77.2 % WP, Impose 61.8 % WG, Phostrol 53.6 % SL, RidomilGold $_{plus}$ 42.5 % WP and Roxyl Plus 86.9 % WP were evaluated for its effect on growth of *P. atrosepticum* on King's B agar plates. Sterilized filter paper discs (6 mm in diameter) impregnated with fungicide concentration was placed on the surface of plates containing inoculated medium with *P. atrosepticum* (3ml of 10^8cfu /ml inoculum in 250 ml capacity flask). The filter paper disc impregnated with 20µl of the fungicide (5000ppm), along with control disc loaded with sterilized water as a control were prepared. Three replicates (plates) per fungicide were used. The incubation was made at 28°C for 48 hr. The inhibition zones around the discs were determined to the nearest mm. The same method was followed to study the efficiency of the afore - mentioned fungicides against *S. scabies* after incubation at 30°C for 3 days.

Determination of MIC :

Minimum inhibitory concentration (MIC) was determined using dilution technique (Radaelli *et al*. 2016). Sterilized YPG broth medium (g/l 5.0 yeast extract; 5.0 peptone; 10.0glucose; distilled water) supplemented with 0.15% (w/v) agar was used. Dilutions of each fungicide in YPG broth were added to YPG medium (3ml / tube) inoculated with either *P. atrosepticum* or *S. scabies*. Total volume of each tube was 4ml and containing 2.5 X 10^7 cfu / ml of either *P. atrosepticum* or *S. scabies*. Final desired fungicide concentrations as 6000, 5000, 4000, 3000, 2000, 1000, 500 and 250ppm were prepared. Tubes of YPG medium and fungicides were used as negative controls. Tubes of YPG medium containing 2.5 X 10^7 cfu / ml inoculum of either *P. atrosepticum* or *S. scabies* were used as a positive control treatment .The tubes were incubated at 30°C for 48 h. Then, 50 µl of resazurin (indicator of microorganism growth) at concentration 0.5 mg/ml in sterile water were added to the tubes with a gentle shaking. After 5h of incubation at 28°C, the carrier materials of fungicide were completely deposited and the MIC was determined. MIC is the lowest Fungicide concentration when no change occurs in resazurin color from blue to pink. Three replicates were considered.

Pot experiments:

Evaluation of the fungicidal potential against blackleg and common scab diseases

Pots (20 cm in diam.) filled with soil mixture (sand: clay: compost at 2: 1 1: ratio) were artificially infested with bacterial pathogens. A single baby tuber (spunta variety) per pot was planted. Seed tubers were kindly obtained from potato brown rot project (PBRP), ARC). Two times fungicide preparations were drenched to irrigate the plants. The first application date was performed at planting and the second after 40day of the first. Control plants (with non-fungicide applications) were considered in each experiment. The treatments were arranged in a completely randomized design.

With regard to black leg disease, two doses (3.0 and 4.0 g/l) of the fungicide Controller 77.2 % WP were used against *P. atrosepticum*. Soil mixture was irrigated with water and after 48 h the soil was drenched with the suspension (10^8cfu/ ml) of *P. atrosepticum* at the rate of 100 ml/pot. Thereafter (72 h of soil infestation) the tubers were planted and then fungicide applications were performed. Five replicates (15 pots / replicate) per treatment were used. Blackleg symptoms on plants were observed visually during 90 days after seeding. Treatment efficacy was estimated as the percent of disease decrease as followed:

Disease decrease % = [(Percent of diseased plants in control- Percent of diseased plants in treatment)/Percent of diseased plants in control) X 100.

With regard to common scab caused by *S. scabies*, two fungicide doses (3.0 and 4.0 g/l) of Controller 77.2 % WP, IMPOSE 61.8 % WG and RidomilGold $_{plus}$ 42.5 % WP were tested. Vermiculite (250 gm) inoculum containing 10^8 CFU of *S. scabies* prepared as described by Wanner *et al*. (2007) was added to the potting mix. Vermiculite (250 gm) without *S. scabies* was added to control treatment. The tubers were planted followed by fungicide treatments, with 5 replicates (3 pots each). Fungicide efficacy was determined visually by estimating the percent of scabby surface of tubers after harvest (100 day after seeding). Scab index which is a measure of scabby surface area was determined as described by McGregor and Wilson (1966) with a modification in tubers classes. The tubers were divided into five classes (class1: Clean tubers, class 2: trace to 10 % of the surface area scabbed, class 3:11 to 25 % of the surface area scabbed, class 4: 26 to 50 % of the surface area scabbed, class 5: more than 50 % of the surface area scabbed).The scab index was calculated by multiplying the number of tubers in classes 1, 2, 3, 4 and 5 by 0, 1, 2, 3 and 4 respectively, summing the products,dividing the sum by the product of four times the total number of tubers, and then multiplying the quotient so obtained by 100 to give the scab index. A scale of 0 for clean tubers to 100 for severely scabbed tubers was resulted. Percent of disease reduction was estimated as followed:

Disease decrease % = [(scab index in control-scab index in treatment)/scab index in control) X 100.

Determination of fungicide residues in potato tubers after harvest:

Sampling

Samples of potato tubers were randomly taken from 4.0 g/l fungicides treatments immediately after harvest and send to the Pesticide Residues and

Environmental Pollution Research Department, Central Agricultural Pesticide Laboratory (CAPL), Dokki, Giza, for analysis.

Determination of copper compound residues in tubers:

Sample preparation, extraction and determination of copper compounds residues (copper oxychloride and copper hydroxide) by Atomic absorption spectrophotometer in the presence of air and hollow cathode lamp for copper were performed according to methods described by Schuller and Coles (1979).

Determination of metalaxyl and cymoxanil residues in tubers:

Sample preparation, extraction and determination of residues were performed according to EN 15662 (2009) using Agilent 1100 HPLC system, with photodiode array detector. The chromatographic column was 150x4.6mm id, x 5µm film thicknesses ODS. Flow rate of mobile phase (Acetonitrile 35% +Methanol 65%) was 1 ml/min and injection volume was 20µl. Detection wave length was set at 205 and 230nm for metalaxyl and cymoxanil respectively. The retention time of metalaxyl and cymoxanil were 2.87 and 2.78 min, respectively.

Determination of dimethomorph and metiram: residues in tubers:

Sample preparation, extraction and determination of residues were performed according to Bonnechere *et al*. (2012) using Agilent series6890N gas chromatograph (GC), equipped with electron capture detector (ECD).The column was PAS-5,(30 m x0.25 mm x0.25µm film thicknesses).The injection port temperature was 290 °C for dimethomorph and 200 °C for metiram. The Initial temperature 200°C(for 2 min) up to 280°C(for 5 min) for dimethomorph and 50°C (for 5 min)for metiram .The detector temperature was 300°C for dimethomorph and 250 °C for metiram. The carrier gas was nitrogen at a flow rate of 3 ml/min.

Statistical analysis:

Completely randomized design was used in all experiments .Collected data were subjected to analysis of variance. Comparisons among treatment means were made using Duncan multiple range test at 0.05 level of probability using software package Costat (version 6.4, CoHort Software, USA)

RESULTS

Effect of tested fungicides on *P. atrosepticum* and *S. scabies in vitro*:

Antimicrobial activity of Controller 77.2 % WP, Impose 61.8 % WG, Phostrol 53.6 % SL, RidomilGold $_{plus}$ 42.5 % WP and Roxyl Plus 86.9 % WP (5000 ppm)were tested against *P. atrosepticum* and *S. scabies in vitro* using agar disc diffusion assay on King's B agar plates. The growth of *P. atrosepticum* was only affected by the fungicide Controller 77.2 % WP which contains dimethomorph, copper oxychloride and cymoxanil. Controller 77.2 % resulted in an inhibition zone 23.3 mm against *P. atrosepticum* while the other fungicides did not have any inhibitory effect on the growth of the bacterium. In case of *S. scabies*, Controller 77.2 % WP, Impose 61.8 % WG (cymoxanil and metiram) and Roxyl Plus 86.9 % WP (metalaxyl and copper hydroxide) showed significant effect and formed inhibition zones 38.0, 23.0 and 24.3 mm , respectively compared to Ridomil Gold plus 42.5 % WP (mefenoxam and copper oxychloride) which formed inhibition zone 8.3 mm, while Phostrol 53.6 % SL (Phosphorus acids salts) did not inhibit the growth of the bacterium (Table, 2 and fig., 1).

Table 2. Effect of Fungicides (5000) on *P. atrosepticum* and *S. scabies in vitro*

Fungicide	**Active ingredients**	**Inhibition zone(mm)**	
		P. atrosepticum	***S. scabies***
Controller 77.2 % WP	Dimethomorph 6.0 % w/w + Copper oxychloride 67.0 % w/w + Cymoxanil 4.2 % w/w	23.3^{a}	38.0^{a}
Impose 61.8 % WG	Cymoxanil 57.0% w/v + Metiram 4.8 % w/v	00.0^{b}	23.0^{c}
Phostrol 53.6 % SL	Phosphorus acids salts 53.6 % w/v	00.0^{b}	00.0^{e}
RidomilGold $_{plus}$ 42.5 % WP	Mefenoxam 2.5 % w/w + Copper oxychloride 69.0 % w/w (= 40 % metallic copper)	00.0^{b}	8.3^{d}
Roxyl Plus 86.9 % WP	Metalaxyl 15 % + Copper hydroxide 53.9 % (= 35 % metallic copper)	00.0^{b}	24.3^{b}
Control	-	00.0^{b}	00.0^{e}

Means in the same column shared a letter are not significantly different ($p \leq 0.05$).

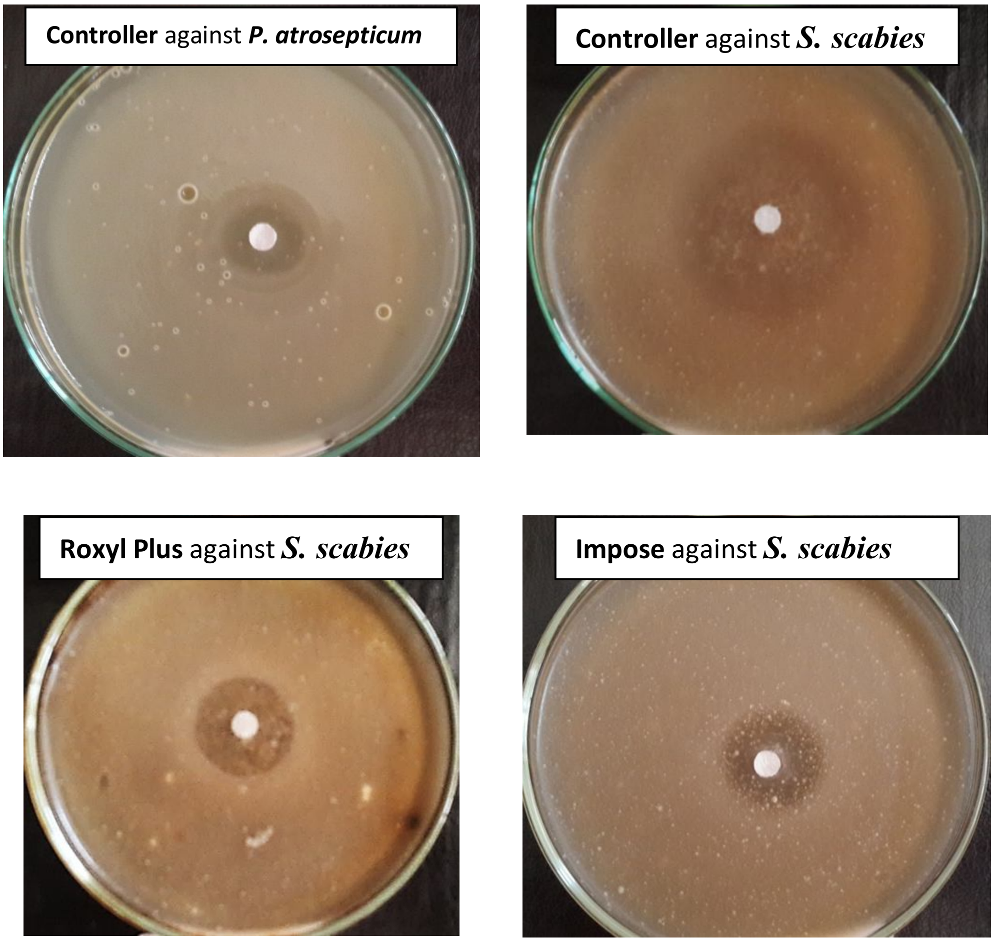

Fig. 1. Effects of fungicides (5000 ppm) on growth of *P. atrosepticum* and *S. scabies*.

MIC of Fungicides:

The MICs of Controller 77.2 % WP were determined for *P. atrosepticum* and *S. scabies* and the MIC value When no colour change of resazurin from blue to pink (unviable cells) was 4000 ppm for the two bacterial species.On the other hand, the MICs of IMPOSE 61.8 % WG and Roxyl Plus 86.9 % WP were determined for *S. scabies* and the values were 4000 and 3000ppm respectively (Table, 3.).

Table 3. minimal inhibitory concentration (MIC) of Fungicides against *P. atrosepticum* and *S. scabies*

Fungicide	Active ingredients	MIC (ppm)	
		P. atrosepticum	***S. scabies***
CONTROLLER 77.2 % WP	Dimethomorph 6.0 % w/w + Copper oxychloride 67.0 % w/w + Cymoxanil 4.2 % w/w	**4000**	**4000**
IMPOSE 61.8 % WG	Cymoxanil 57.0% w/v + Metiram 4.8 % w/v	**ND**	**4000**
Roxyl Plus 86.9 % WP	Metalaxyl 15 % + Copper hydroxide 53.9 % (= 35 % metallic copper)	**ND**	**3000**
Control (-)	-	-	-
Control (+)	-	**NE**	**NE**

ND = Not determined, NE =No effect.

Effect of certain fungicides on blackleg and common scab:

Controller 77.2 % WP the only tested fungicide that showed inhibition of the growth of *P. atrosepticum in vitro* was tested in pots experiment. Data in Table (4) indicated that application of Controller 77.2 % WP against blackleg disease significantly decreased incidence of blackleg of potato plants from 86.7 % in control to 46.7 and 30.7 % when 3.0 and 4.0 g/l of the fungicide were used, respectively. The disease reduction was, therefore, significantly increased from 45.9 to 64.3% by increasing the fungicide dose from 3.0 to 4.0 g liter.

Table 4. Effect of Controller 77.2 % WP on Blackleg incidence

Treatment	Active ingredients	Percent of diseased plants (Blackleg)%	Disease decrease %
Controller 77.2 % WP (3.0 g/l)	Dimethomorph 6.0 % w/w + Copper oxychloride 67.0 % w/w + Cymoxanil 4.2 % w/w	46.7^{b}	45.9^{b}
Controller 77.2 % WP (4.0 g/l)		30.7^{c}	64.3^{a}
Control (+)	**-**	86.7^{a}	00.0^{c}
Control (-)	**-**	00.0^{d}	-

Means in the same column shared a letter are not significantly different ($p \leq 0.05$).

Application of 3.0 and 4.0 g/l of Controller 77.2 % WP, Impose 61.8 % WG and Roxyl Plus 86.9 % WP as soil drench in pots significantly decreased scab index compared to control treatment. Scab index was decreased from 40.1 to 28.0 and 22.7, 17.6 and 17.0, and 15.9 and 12.9 with the above treatments, respectively. The respective decreases in common scab were 28.6 , 43.5% for Controller 77.2 % WP. Although the Controller 77.2 % WP fungicide gave the best antibacterial activity against *S. scabies* in the laboratory, it did not show good results in pots as shown in table (5). Roxyl Plus 86.9 % WP gave the best common scab decrease followed by Impose 61.8 % WG and Controller 77.2 % WP, respectively.

Table 5. Effects of different fungicides on common scab severity

Treatment(g/l)	Active ingredients	scab severity %	Disease decrease %
Controller 77.2 % WP(3.0 g/l)	Dimethomorph 6.0 % w/w + Copper oxychloride 67.0 % w/w + Cymoxanil 4.2 % w/w	28.8[b]	28.6[e]
Controller 77.2 % WP (4.0 g/l)		22.7[c]	43.5[d]
Impose 61.8 % WG(3.0 g/l)	Cymoxanil 57.0% w/v + Metiram 4.8 % w/v	17.6[d]	56.2[c]
Impose 61.8 % WG(4.0 g/l)		17.0[de]	57.8[bc]
Roxyl Plus 86.9 % WP (3.0 g/l)	Metalaxyl 15 % + Copper hydroxide 53.9 % (= 35 % metallic copper)	15.9[e]	60.5[b]
Roxyl Plus 86.9 % WP(4.0 g/l)		12.9[f]	67.9[a]
Control (+)	-	40.1[a]	00.0[f]
Control (-)	-	00.0[g]	-

Means in the same column shared a letter are not significantly different ($p \leq 0.05$).

Residues of used fungicides in potato tuber:

Residues of the active ingredients of fungicides Controller 77.2 % WP ,Impose 61.8 % WG and Roxyl Plus 86.9 % WP in harvested potato tuber 60 day after the latest application , at rate of 4 g/l, on potato plant in pot experiments were shown in Table (6). The maximum residue level of dimethomorph, copper oxychloride, cymoxanil, metiram, metalaxyl and copper hydroxide according to standards of the European Commission (EU Pesticides database**,** are 0.05, 5.00, 0.01, 0.30, 0.05 and 5.00 mg/kg respectively. The initial deposits of cymoxanil, metiram and metalaxyl were undetectable on harvested potato tuber (after 60 day of the last application) when the first application was performed during planting and the other after 40 day of the first application at the rate of 4 g/l as soil drench treatment in pot experiments. The initial deposits of dimethomorph, copper oxychloride and copper hydroxide were

0.025, 0.186 and 0.414 mg/kg respectively. Content of copper in the potato tubers of control treatment was 0.180 mg/kg.

Table 6. Residues of Controller, Impose and Roxyl Plus in harvested potato tuber (mg/kg)

Fungicide treatment (g/l)	**Active ingredients**	**Maximum residue level (MRL) mg/kg***	**Residue concentration(mg/kg)onpotato tuber**	
			Treatment	**Control**
CONTROLLER (4.0 g/l)	Dimethomorph	0.05	0. 025	UND
	Copper oxychloride	5.00	0.186	0.180
	Cymoxanil	0.01	UND	UND
IMPOSE (4.0 g/l)	Cymoxanil	0.01	UND	UND
	Metiram	0.30	UND	UND
Roxyl Plus (4.0 g/l)	Metalaxyl	0.05	UND	UND
	Copper hydroxide	5.00	0.414	0.180

*http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN
UND= undetectable

DISCUSSION

In the present study, the antimicrobial potential of Controller, Impose, Phostrol, Ridomil Gold $_{plus}$ and Roxyl Plus was tested *in vitro*. Results showed that the growth of *P. atrosepticum* was affected by the fungicide Controller 77.2 % WP (dimethomorph, copper oxychloride and cymoxanil) while the other fungicides did not exert any effect on the growth of the bacterium. In case of *S. scabies*, Controller, Impose (cymoxanil and metiram) and Roxyl Plus (metalaxyl and copper hydroxide) showed high effect with a significant difference compared to Ridomil Gold plus (mefenoxam and copper oxychloride). The Phostrol (Phosphorus acids salts) did not inhibit the growth of the bacterium. In this regard, Kokoskova (1992) tested a number of bactericides and fungicides against plant pathogenic bacteria of different genera *in vitro* and found that Kuprikol 50 (copper oxychloride), Champion 50 (copper hydroxide), Kocide 101 (copper hydroxide), Sandofan C (oxadyxil, copper oxychloride) and Curzate K (cymoxanil, copper oxychloride) were effective at varying degrees . Aliette (fosetyl Al) and Ridomil Plus 48 WP (methalaxyl, copper oxychloride) on the other hand, were ineffective. Norman *et al.* (2006) found that Phosphorous acid inhibited the growth of *R. solanacearum in vitro*, acting as bacteriostatic compound in the soil and protecting plants from infection. The antibacterial activity of Miedzian 50 WG (50% copper oxychloride), Ridomil MZ Gold 68 WG (3.8% metalaxyl-M and 64%, mancozeb), Euparen Multi 50 WG (50% tolylfluanid), Captan 80 WG [80% N-(captan)], Dithane Neotec 75 WG (75% mancozeb) against *E. amylovora*, *X. arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis*, *P. syringae* pv. *syringae*, *Ag. tumefaciens in vitro* showed that all tested bacteria were inhibited with metalaxyl-M with mancozeb, mancozeb alone, and copper oxychloride while Tolylfluanid did not inhibit any of the tested bacteria (Mikicinski *et al.*,2012). From cumulative results we can observe that the presence of copper oxychloride with dimethomorph, and cymoxanil in the fungicide composition is more efficient in inhibiting the growth of *S. scabies in vitro* than presence of copper oxychloride with mefenoxam. Also, phosphorous acid does not inhibit the growth of all bacterial species *in vitro,* despite of its ability to inhibit growth of *R. solanacearum*.

It has been reported that the *in vitro* MICs values of aluminum salts, metabisulfite, trisodium phosphate, sodium carbonate, and sodium propionate for *P. carotovorum* subsp*. carotovorum* and *P. atrosepticum* were ≤10 mM, comparing to 100 mM for sodium benzoate and potassium sorbate (Yaganza *et al*., 2014). In the present study, the MIC of Controller 77.2 % WP for *P. atrosepticum* isolate was reported to be 4000 ppm. In literature the concentrations of dazomet, dimethomorph plus dithianon, mancozeb, oxadixyl, streptomycin sulfate, validamycin A, chloramphenicol, cycloheximide, nalidixic acid, and neomycin sulfate were tested against two isolates of *S. scabies*. A remarkable variation in sensitivity to antibiotics and / or pesticides was observed between the two isolates. *S. scabies* DSMZ 40962 was highly sensitive to all the tested compounds except cycloheximide and validamycin and MICs ranged from10 to 100 ppm, while *S. scabies* ATCC49004 was resistant to most tested compounds and MIC ranged from 50 to >5000 ppm(Lee *et al*.,2004). MICs of Controller 77.2 % WP Impose 61.8 % WG and Roxyl Plus 86.9 % WP for our *S. scabies* isolate were 4000, 4000 and 3000 ppm, respectively.

Application of Controller 77.2 % WP (Dimethomorph plus Copper oxychloride plus Cymoxanil) against blackleg significantly decreased its incidence from 86.7 % in control to 46.7 and 30.7 % with 3.0 and 4.0 g/l, respectively. With regard to protective effect of fungicides on other crops the Miedzian 50 WG (50% copper oxychloride), Ridomil MZ Gold 68 WG (3.8% metalaxyl-M and 64%, mancozeb), Euparen Multi 50 WG (50% tolylfluanid), Captan 80 WG [80% N-(captan)] ,and Dithane Neotec 75 WG (75% mancozeb) against fire blight on apple and pear, bacterial canker on sweet cherry, and crown gall on sunflower revealed that only copper oxychloride had a protective activity (Mikicinski *et al*.,2012). Although the bacteria do not contain sterols, sterol-inhibiting fungicides have indirect side effects on bacteria .The activity of the bacteria involved in the N cycle is affected by the dimethomorph fungicide, with effect on nitrification and ammonification, through its different effect on different bacterial ecotypes and changes in bacterial community structure (Yang *et al*., 2011).Moreover, it is shown in this work that scab index was decreased from 40.1 to 28.0 and 22.7 for controller, and a greater decrease was recorded for Impose and Roxyl plus either at 3.0 and 4.0 g/l . *In vitro* antibacterial properties of some fungicides did not correspond with their potential protective on the plant in the *in vivo* (Mikicinski *et al*., 2012). Although the Controller 77.2 % WP gave the best activity against *S. scabies* in the laboratory, it did not show good results in pots, where Roxyl Plus 86.9 % WP (Metalaxyl plus Copper hydroxide) gave the best disease decrease followed by Impose 61.8 % WG (Cymoxanil plus Metiram) and Controller 77.2 % WP, respectively. Some research suggested mancozeb and captan as seed treatments for control common scab and others reported no effects on incidence and severity when thiophanate-methyl, mancozeb, captan, metiram, chlorothalonil and fluazinam were used as a seed treatment (Stead and Wale, 2004). Many experiments confirmed the efficacy of copper compounds against plant pathogenic bacteria. Most bacterial plant diseases practically influenced by copper compounds but the success rate is rarely satisfactory because the effect is protective and requires many sprays which leading to higher costs and phytotoxic effects on plants especially on fruits (Egli and Sturm, 1981).

Fungicides are highly harmful to human and animal health, if found in food above approved residue listes. In our study, the initial deposits of cymoxanil, metiram and metalaxyl were below the maximum residue level and undetectable on harvested potato tuber. Also, the initial deposits of dimethomorph, copper oxychloride and copper hydroxide were 0. 025, 0.186 and 0.414 mg/kg respectively and below the maximum residue approved. Copper is naturally found in soil with a content of 2.0 to 60.0 mg/kg without hazardous effect, while copper in arable land is estimated to be between 5.0 and 30.0 mg/ kg (Pavlovic, 2011). Content of copper in the potato tubers in our control treatment, however, did not exceed 0.180 mg/ kg.

REFERENCES

1. Bonnechere A., Hanot V., Jolie R., Hendrickx M., Bragard C., Bedoret T., van Loco J. 2012.Effect of household and industrial processing on levels of five pesticide residues and two degradation products in spinach. Food Control, 25:397–406.
2. Czajkowski R., Pérombelon M. C. M., van Veen J. A. and van der WolfJ. M. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and Dickeya species: a review. Plant Pathology, 60: 999–1013.
3. Egli T. and Sturm E. 1981.Bacterial plant diseases and their control. In: Wegler, R. (ed.), Chemie der Pflanzenschutz and Schadlingsbekampfungsmittel, Berlin, Springer-Verlag, Vol. 6, pp. 345–388.

4. EN 15662, 2009. Foods of plant origin, determination of pesticide residues using GC-MS and/or LC-MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS-method, English version DIN EN15662 Edition: 2009-02.
5. Holm A. L., Rivera V. V., Secor G. A. and Gudmestad N. C. 2003. Temporal sensitivity of *Alternaria solani* to foliar fungicides. American Journal of Potato Research, 80:33-40.
6. Hu J. H., Hong C. X., Stromberg E. L. and Moorman G. W. 2008. Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. Plant Pathology, 57, 728–736.
7. Keinath, A.P. 2010. Potassium phosphite mixed with other fungicides reduces yield loss to downy mildew on collard.Plant Management Network News*,* doi: 10.1094/PHP-2010-0823-01-RS.
8. Kokoskova B. 1992. The efficiency of chemicals against the plant pathogenic bacteria *in vitro*. Ochrana Rostlin, 28:207-214.
9. Lee, H.B., Cho, J.W., Park, D.J., Li, C.T., Ko, Y.H., Song, J.H., Koh, J.S., Kim, B.J. and Kim, C. J. 2004. *In vivo* screening for biocontrol agents (BCAs) against *Streptomyces scabiei* causing potato common scab. The Plant Pathology Journal, 20: 110–114.
10. Magarey P.A., Wicks T.J. and Wachtel M.F. 1990.Phosphonic (phosphorous) acid controls *Plasmopara viticola* the cause of downy mildew of grapevines. Australasian Plant Pathology, 19: 126–127.
11. Malhat F. 2012. Persistence of metalaxyl residues on tomato fruit using high performance liquid chromatography and QuEChERS methodology. Arabian Journal of Chemistry, http://dx.doi.org/10.1016/j.arabjc.2012.12.002.
12. McGregor A. J. and Wilson G. C. S.(1966).The influence of manganese on the development of potato scab.Plant and Soil,25(1): 3-16
13. Mikicinski A., Sobiczewski P. And Berznski S. (2012). Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. Journal of Plant Protection Research, 52(4):467-471.
14. Norman D. J., Chen J., Yuen J. M. F., Mangravita-Novo A., Byrne D. and Walsh L. 2006. Control of bacterial wilt of geranium with phosphorous acid.Plant Dis. 90:798-802.
15. Pavlovic M. 2011.A review of agribusiness copper use effects on environment. Bulg. J. Agric. Sci., 17:491–500
16. Radaelli, M., da Silva B. P.,Weidlich L.,Hoehne L., Flach A., da CostaL. A.M. A. and Ethurb E. M. 2016. Antimicrobial activities of six essential oils commonly used as condiments in Brazil against *Clostridium perfringens*. Brazilian Journal of Microbiologym .47:424-430.

17. Raoul des Essarts Y., Cigna J., Quetu-LaurentA., Caron A., Munier E., Beury-Cirou A. , Helias V. and Faure D. 2016. Biocontrol of the potato blackleg and soft rot diseases caused by *Dickeya dianthicola*. Applied and Environmental Microbiology, 82: 268-278.
18. Rusjan D. 2012. Copper in Horticulture. In: Dharumadurai D. (ed) Fungicides for Plant and Animal Diseases. ISBN: 978-953-307-804-5, In Tech, https://cdn.intechopen.com/pdfs-wm/26033.pdf
19. Sakr S. A. and Shalaby S. Y.2012.Metiram-induced histological and histochemical alterations in Liver and kidney of pregnant mice,Life Science, 9(1):71-76.
20. Schuller P. L. and Coles L. E. 1979. The determination of copper in foodstuffs (International Union of Pure and Applied Chemistry, Applied Chemistry Division, Commission on Food Contaminants). Pure Appl. Chem. 51:385.–392.
21. Sisler H.D. and Ragsdale N.N. 1985. Mode of action and selectivity of fungicides. In: Hilton JL (ed) Agricultural chemicals of the future. Rowman and Allanheld, Totowa, NJ, pp 175–188.
22. Stead D. and Wale S. 2004. Non-water control measures for potato common scab. Oxford, British Potato Council.
23. Wanner, L. A. 2007. A new strain of *Streptomyces* causing common scab in potato. Plant Disease . 91:352-359.
24. Yaganza E.S., Tweddell R.J., Arul J. 2014. Postharvest Application of Organic and Inorganic Salts to Control Potato (*Solanum tuberosum* L.) Storage Soft Rot: Plant Tissue−Salt Physicochemical Interactions. Journal of Agricultural and Food Chemistry, 62: 9223-9231.
25. Yang C., Hamel C., Vujanovic V. and Gan Y. 2011. Fungicide: modes of action and possible impact on non-target microorganisms. ISRN Ecol 2011:130289. doi:10.5402/2011/130289.
26. Ziogas B. N. and Davidse L. C. 1987. Studies on the mechanism of action of cymoxanil in *Phytophthora infestans* .Pesticide Biochemistry and Physiology, 29:89-96.

تقييم فعالية بعض مبيدات الفطريات في مكافحة الساق السوداء والجرب العادي على البطاطس

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تم تحديد القدرة المضادة للبكتريا لكل من كنترولار،امبوز،فوستيرول ، ريديوميل جولد وروكسيل بلس على البيكتوبكتيريم اتروسيبتيكم *Pectobacterium atrosepticum* والاستربتوميسس سكابس *Streptomyces scabies* . لم يظهر التأثيرالقاتل ضد البيكتوبكتيريم اتروسيبتيكم *P. atrosepticum* إلا من قبل المبيد الفطري كنترولار. الاستربتوميسس سكابس *S. scabies* تأثر بشدة من قبل كنترولار ، امبوز و وروكسيل بلس مقارنة مع ريديوميل جولد و فوستيرول حيث لم يمنعا النمو. وجد ان أقل تركيز مثبط (MIC) من كنترولار هو 4000 جزء في المليون لكل من البيكتوبكتيريم اتروسيبتيكم *P. atrosepticum* و الاستربتوميسس سكابس *S. scabies* . من ناحية أخرى ، وجد ان أقل تركيزات قاتلة (MICs) من امبوز و روكسيل بلس هي 4000 و3000 جزء في المليون على التوالي لاستربتوميسس سكابس *S.scabies*. استخدام كنترولار ضد مرض الساق السوداء خفض بشكل ملحوظ حدوث الساق السوداء في نباتات البطاطس المزروعة في الاصص. استخدام كنترولار ، امبوز و وروكسيل بلس عند 3.0 جرام / لتر لمكافحة الجرب العادي خفض بشكل معنوي من المرض . تم الحصول على افضل النتائج عند استخدام 4.0 جرام / لتر. كانت متبقيات مكونات هذه المبيدات الفطرية في درنة البطاطس بعد 60 يومًا من الاستخدام أقل من مستوى الحد الأقصى المتبقي(MRL) المسموح به.