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Monitoring Biodegradation of Gramonol Herbicide by *Streptomyces scabies*

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ABSTRACT

The biodegradation behavior of pesticides on and in various edible plants was studied by many others. They concluded that the current levels of the pesticides in surface water and plants do not constitute an acute toxicity hazard to man and animals on a short-term basis, but as a result of their accumulation in the main body, it causes a hazard of long-term exposure. Visual observation by light and electron microscopes of four *Streptomyces* spp. isolated from rhizosphere potato plants showed differentiation in mycelium and conidia spores as well as a variable diffusible pigment in a selective medium. The four *Streptomyces* spp. were identified as *Streptomyces scabies* by VITEK 2 system. The potential of granola herbicide degradation in relation to *S. scabies* isolates detected by polymerase chain reaction (PCR). Four *S. scabies* isolates showed different potential abilities to degrade the gramonol in Lab. and in soil in the course of 5 days of incubation and 35 days post herbicide treatment respectively. It is clear that *S. scabies* isolates St_4 was more active in gramonol degradation than the other isolates. Dehalogenase gene was detected by PCR in DNA genome of 4 isolates. It was found differences among isolates related to fragment density and size. Residual gramonol determined by GLC after 10, 20 and 30 days. It was observed the degradation rate increased with increased time as well as a small amount of gramonol residue was observed in non-sterilized soil compared with sterilized soil in relation to increasing the count of total microorganisms. On the other hand, the effect of gramonol on Streptomyces isolates growth was non-observed.

INTRODUCTION

Biological control of potato common scab with Rare Isatropolone compound produced by plant growth promoting Streptomyces A1RT (Sarwar, *et al.*,2018) Plant Natural Products as Antimicrobials for control of *Streptomyces scabies* : A causative agent of the common scab disease (Gutierrez *et.al.* 2022 *and Wikipedia ,2023).*

Liu,*et.al.* (2023) findings characterize a multifunctional regulatory protein, SCAB_Lrp, that controls secondary metabolism, pathogenicity, and sporulation in *S. scabies* and provide new insights into the complex regulatory network that modulates thaxtomin phytotoxins in pathogenic Streptomyces.

The biodegradation behavior of pesticides on and in various edible plants was studied by many others. However, this type of study is carried out under the local conditions of every country. They concluded that the current levels of the pesticides in surface water and plants do not constitute an acute toxicity hazard to man on a short-term basis, but as a result of its accumulation in the main body, it causes a hazard of long-term exposure. On the other hand, some studies were published about the methods of removing pesticides from soil and water by biodegradation using microorganisms (Yakato *et al.,* 1987 and Dahrog *et al.,* 2006) So far haloalkinase Dehalogenase is the early enzyme known to be capable of direct hydrolytic dehalogenation of chlorinated and brominates hydrocarbons without the requirements for enzymes or oxygen.The enzyme of *Xanthomanas autotrophic* and Rhizobium sp. constitutively expressed 2 to 3 % of the solute cellular protein (Keuning *et al.,* 1985 and Azhar, 2009) up to 30 % (Janssen *et al.,* 1989). It has a remarkably broad substrate range which includes terminally halogenated alkenes with chain lengths of up to 4 carbons for chlorinated and up to at least two carbons for brominate alkenes.Another haloalkinase Dehalogenase of a broad substrate range has been formed in gram-positive haloalkinase, utilizing bacteria (Janssen *et al.,* 1989). The objective of the present study was to clarify the degradation of gramonol herbicide by Streptomyces sp. under laboratory and pot-experiments.

MATERIALS AND METHODS

Isolation and Identification of *Streptomyces* Isolates:

Rhizosphere soil samples (salty clay soil) under cultivated potato plants were collected from different locations in Egypt according to the procedures described by Johnson *et al*., (1960). In this method, soil samples were collected by sterilizing a hand corer at a depth of 15 cm from different regions, in clean plastic bags. The collected samples were air-dried and ground then mixed with $CaCO_3$ and followed by sieving in a 4 mm mesh screen. One gram of each prepared soil was stirred in 100 ml sterile water and serial dilution until 10^{-6}. One ml of each dilution was spread on a petri dish containing starch nitrate agar medium (Tadashi, 1975). The dishes were incubated for 7 days at 28ºC till *Streptomyces* colonies appeared. The purification was achieved according to (Kuster and Williams, 1964) by picking up unique single identical morphological *Streptomyces* colonies. Twenty purified *Streptomyces* isolates were sub-cultured on a specific medium and stored at 4ºC.

Light microscopy was used to determine spore chain morphology cultured on starch nitrate agar medium by Petri-dishes as described in the international *Streptomyces* project (ISP). The sterile media were inoculated in a cross-hatched pattern with the selected *Streptomyces* after cover slip insertion at º45 on starch nitrate agar and incubated at 28 ºC. After 7, 14 days of the incubation period, one of the coverslips was withdrawn to be examined for spore chain morphology under light microscopes. Spore chain shape was described according to the categories of Pridham *et al*., (1958).

Transmission electron microscopy (TEM) was used to determine The sporulating surface of spore chains by the spore-print technique according to (Tresner *et al*., 1961). Carbon-coated grids were gently pressed over the sporulating surface of starch nitrate agar cultures. The spore chains, which adhere to the coated surface of the grids, were observed and photographed using a JOEL-JEM-1010 transmission electron microscope (Electron Microscope Unite, Al-Azhar University, Cairo).

Different media cultures were used to investigate the cultural properties of *Streptomyces* isolates. The cultural characteristics were determined for 14 days cultures maintained on the following media; starch- nitrate agar (Tadashi,1975), glycerol-asparagine agar (Pridham and Lyons, 1961), oat-meal agar (Kuster,1959a & b), yeast malt extract agar (Pridham, 1965), glycerol-nitrate agar (Waksman, 1961), inorganic starch agar (Kuster, 1959a &b), nutrient-agar (Shirling and Gottlieb, 1966), glucose-asparagine agar (Waksman, 1961), glucose- nitrate agar and sucrose- nitrate agar (Waksman, 1961)

Color of sporulated aerial mycelium properties was expressed as color of colony (color of sporulated aerial mycelium), substrate mycelium (reverse side of colony) and diffusible pigments other than melanoidin and its sensitivity to change in pH of the medium were recorded (Shirling and Gottlieb, 1966).

Determination of melanin pigment was carried on selective media as described by Shirling and Gottlieb, (1966). It was determined by incubation of *Streptomyces* isolates on peptone yeast extract iron agar (ISP-6) tyrosine agar (Gordon and Smith, 1955) and tryptone-yeast extract broth (Pridham and Gottlieb, 1948) which encourage pigment production for 4 days at 28^{o}C. Typically, the production of brown or dark brown melanin pigment at early stages of growth indicated positive melanin production activity.

Identification of *Streptomyces* isolates was done by VITEK2 system applied Card Installed VITEK 2 ® Systems Version: 08.01 for Gram-positive (GP) spore-forming (McFarland Turbidity Range 0.50-0.63). Also, contain an antimicrobial susceptibility test that can determine the minimum inhibitory concentration (MIC) against microorganism. (Shetty, *et al.*,1998 and Funke, *et al.*,1998). The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substance. The cards are incubated and interpreted automatically.

Preparation Suspension Pure Culture:

A disposable bacterial needle used to transfer a single colony of *Streptomyces* isolates pure cultures and suspended in 3.0 ml of sterile saline (aqueous 0.45% to 0.50%NaCl, pH4.5 to7.0) in a 12x75 mm clear polystyrene test tube. The turbidity was adjusted according to (McFarland Turbidity Range 0.50-0.63 and measured using a turbidity meter (DensiChek).

Inoculation the inoculated cards were *Streptomyces* isolates suspensions using an integrated vacuum apparatus. A test tube containing *Streptomyces* isolates suspensions was placed into an especial rack (cassette) and the identification card was placed in the neighbor in gloat while inserting the transfer tube into the corresponding suspension tube.

Antibiotic Sensitivity Test:

(Vitek 2 system was used to antibiotic sensitivity test): Cefaclor, Cefotaxime, Cefoperazone, Cefepime, Clindamycin, Imipenem, Doxycycline, Levofloxacin, Ciprofloxacin, Amikacin, Sulphamethoxazole /Trimesoprim, Azithromycin, Ampicillin, Amoxicillin/ Clavulinic acid, Pipracillin /Tazobactam and Nitrofurantoin) used MIC Interpretation Guideline: Global CLSI-based (Cursino *et al.*, 2005).

Antibiosis: Microbial pathogenic used for antibiosis kindly provided from the Cairo MARCN Fac. of Agric. Ain Shams University, Egypt. The antimicrobial activity of four *Streptomyces* spp was assessed by the cork-borer method as follows: *Bacillus cerieus*, *Pseudomonas aeruginosa*, were inoculated on nutrient agar medium as well as *A. flavus*, and *Candida albicans* inoculated in on malt extract agar medium. The plugs of each of four *Streptomyces* spp. were cut out by the cork-borer and placed on the surface of the agenized medium seeded with microbial pathogeneses. The plates were incubated at 37^{0}C for 24h (bacteria), 30^{0}C for 48 h and 25^{0}C for 72 h (Fungi). This method is based on the microbial growth inhibition zone of a biologically active four *Streptomyces* spp.

Lab. Experiment:

Herbicide:

Gramonol 24% liquid E.C. supplied by Imperial Chemical industries limited U.K. (ICI), containing frequent Ion, 100 g/L (1-1 dimethyl, 4-4-bipyridylum ion and Monolinuron), 140 g/L, 3- (4-Chloropenyl) - 1- methoxy-1-methyl urea.

Gramonol herbicide clearing zone: Assay was carried out on starch-nitrate agar medium containing (20 g starch, 2g KP04, 1g KH2P04, 0.5g MgS04, 0.5g NaCl; 3gCaC03; 0.01 FeS04; 1g trace salt solution up to on liter according to Tadashi (1975), and supplemented with 0.5% (V/V) of emulsified gramonol (50%). Each of the four *Streptomyces* isolates was distributed at $1x10^5$ to the surface of Petri dishes, three replicates of each isolate. The Petri dishes were incubated at 37ºC and 65 ± 1% relative humidity. After 5 days, Petri dishes were inspected to estimate the efficacy of *Streptomyces* isolates on the degradation of gramonol herbicide by using the methods according to Dahrog *et al*. (2006).

Potted Experimentally Mottling System:

Greenhouse Experiment:

The soil samples were taken at the 15 cm depth from the soil. It was mixed with organic fertilizer at the ratio 2:5 (W/W) and sterilized at 121ºC / 1 air pressure for 20 min. The sterilized soil was transferred into pots and injected with $5.2x10^4$ of each *Streptomyces* isolate of each pot according to Blazevic and Ederer (1975). One cup of water (200ml) was poured into each pot to raise the moisture of the soil. After three days the pots were sprayed with gramonol 50% WP solution at the recommended concentrations, then 25ml urea (30%) was added to each pot for the purpose of adjusting pH and activating *Streptomycetal* growth. *Streptomycetes* isolates were counted in treated and untreated soil samples at 10-, 20- and 30-days post-soil inoculation. The part of the soil was kept in a freezer up till analysis to determine the concentration of gramonol herbicide.

Determination of gramonol residues: Gramonol herbicide residues were extracted clean up and concentration was determined according to Dahrog, *et al*., (2006)

Soil free from the tested herbicide was used to estimate the rate of recovery by using the previous procedures. The main of the obtained recovery was 90.5%. All the obtained data for the residues of gramonol on treated soil were corrected by using such a rate of recovery.

A series of concentrations, 10, 20, 30, 40, 50 and 60 ng of Afalon analytical standard in 10ml Toluene were prepared to obtain the standard curve A suitable aliquot (5ml) was injected from each concentration.

Determination of dihalogenase activity: *Streptomyces* spp isolates were inoculated into a starch liquid medium and incubated at 28 ± 2ºC for 2 weeks under stirred conditions as described by Tadashi (1975). The developed growth was harvested by centrifugation at 5000 rpm and determined cellular proteins by the Bradford method (1976) using bovine serum albumin (BSA) as a standard protein. The enzyme activity was assayed according to Jensen *et al*. (1987).

Isolation of Genomic DNA:

From *Streptomyces scabies*, cell cultures were harvested by centrifugation. The DNA was extracted using CTBA method as described by Owen and Borman (1987).

Detection of Dihalogenase Gene DNA:

The dihalogenase gene was amplified by polymerase chain reaction (PCR) in 100 ml react mixture containing: 20ml template DNA (25 mg), 0.2 ML taq polymerase, 12.0 µl dNTPs (25 mM of dATP, dTTP, dCTP and dGTP), 3.0 µl $MgCl_2$ (25MM), 30 µl PCR reaction buffer (10X), 20 µl specific primers (Dehalogenase encoding gene) (TF primer tgggcggatttttgggget and TR primer: gtacgaaatggccagcgtcc) and 23.8 µl d H_2O. The PCR program includes one cycle at 94ºC for 2 min and then 30 cycles at 94ºC/30 sec.; 55ºC for 30 sec. and 72ºC for 20 sec. One cycle at 72ºC for 5 min then store in 4ºC final mix of PCR.

DNA amplification was confirmed on agarose 1.5% then stained with ethidium bromide and visualized on UV Tran illuminator and photographed.

RESULTS

Identification of Isolated *Streptomyces scabies*:

Morphological Characteristics:

The four selected Streptomyces isolates out of 25 *Streptomyces* isolates which distinct (color of aerial mycelia, substrate mycelia and reserve color in media) were identified according to cultural growth, morphological, melanin pigment and antibiosis characters following the diagnostic key of Bergey's Mannual (1989). It had been found that the isolates namely St1, St2, St4, & St4 can be identified as *Streptomyces scabies*,

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Culture Growth:

According to the color of the sporulated aerial mycelium the experimental isolates were differentiated into four color, dark grayish series, yellowish series, pale brownish series and green series. The four isolates revealed variable growth rates on different media (Table 1). nutrient agar, glycerol nitrate agar, nutrient agar, glucose asparagine's agar and sucrose nitrate agar media. The four isolates showed diffusible pigment in all experimental media under study

Table 1: Cultural characteristics, aerial & substrate mycelium color and diffusible pigment of *Streptomyces scabies* isolates.

Media	Cultural	*Streptomyces scabies* isolates			
		St scabies 1	*St scabies 2*	*St scabies 3*	*St scabies 4*
Starch Nitrate agar	Aerial color	Black grayish ++++	Yellowish white +++	Brownish-yellow ++++	Greenish white ++++
	Substrate color	Pale greenish	Brownish-yellow	Greenish yellow	greenish
	Diffusible pigment	-ve	-ve	-ve	-ve
Glycerol nitrate agar	Aerial color	Dark grayish +	Yellowish white ++	Brownish-yellow ++++	Greenish white +++
	Substrate color	Pale greenish	Pale yellowish	Greenish yellow	Dark green
	Diffusible pigment	-ve	-ve	-ve	-ve
Inorganic starch agar	Aerial color	Dark grayish +++	Pale brownish ++++	Brownish-yellow ++++	Greenish w hite ++++
	Substrate color	Pale greenish	Pale greenish	Dark brownish yellow	Greenish
	Diffusible pigment	-ve	-ve	-ve	-ve
Nutrient agar	Aerial color	Grayish +	Pale yellowish ++	Yellowish grayish ++	Variable White
	Substrate color	Pale greenish	Pale yellowish	Pale yellowish	Pale yellowish
	Diffusible pigment	-ve	-ve	-ve	-ve
Glucose asparagine agar	Aerial color	Dark grayish +	Brownish grayish ++	Brownish grayish +++	Greenish white ++
	Substrate color	Pale greenish	Pale yellowish	Yellowish brown	Dark green
	Diffusible pigment	-ve	-ve	-ve	-ve
Yeast Malt agar	Aerial color	Greenish grayish ++	Brownish grayish ++	Brownish-yellow ++	Greenish white +++
	Substrate color	Yellowish green	Pale yellowish	Olive greenish	Dark green
	Diffusible pigment	-ve	-ve	-ve	-ve
Glucose nitrate agar	Aerial color	Olive grayish ++	Brownish grayish ++	Brownish-yellow +++	Dark greenish ++++
	Substrate color	Pale greenish	Pale Yellowish grayish	Dark yellowish	Dark green black
	Diffusible pigment	-ve	-ve	-ve	-ve
Glycerol asparagine agar	Aerial color	Olive greenish ++	Brownish gray +++	Pale brownish yellow +++	Dark greenish +++
	Substrate color	Pale greenish	Yellowish grayish	Pale yellowish	Dark green
	Diffusible pigment	-ve	-ve	-ve	-ve
Sucrose nitrate agar	Aerial color	Yellowish greenish +	Pale brownish +++	Brownish-yellow ++++	Dark green ++++
	Substrate color	Pale yellowish	Yellowish green	Greenish yellow	Greenish
	Diffusible pigment	-ve	-ve	-ve	-ve
Oatmeal agar	Aerial color	Green olive +++	Brownish grayish ++	Greenish grayish ++	Pale greenish ++++
	Substrate color	Pale greenish	Yellowish green	Pale yellowish	Dark green
	Diffusible pigment	-ve	-ve	-ve	-ve

Growth: ++++ : Very strong growth , +++ : Strong growth , ++ : Moderate growth + Weak strong,
V: Variable growth

The isolates revealed variable morphology by light and electron micrographs which showed no sporangium formation and differences in conidiophores (Table 2 & Fig 1). In relation to the spore chain were different, spiral short, spiral long, spiral open long, spiral open long and spiral long (Fig 3). The spore mass also differed between isolates (dark grayish, yellowish-white, pale brownish and yellow-brownish). As well as Their surface was also hairy, spiny and smooth. Conidia spores' morphology differed (oval shape, barrel shape) (Table 2).

Table 2: Morphological sporangium characteristics of *Streptomyces scabies* isolates.

Sporangium characters	*St scabies 1*	*St scabies 2*	*St scabies 3*	*St scabies 4*
Conidiophores	6µm	4.5 µm	4 µm	3 µm
Spore mass	Dark grayish	Yellowish white	Yellow brownish	Yellow brownish
Spore surface	Hairy	Smooth	Smooth	Spiny
Spore chain	Spiral short	Spiral long	Spiral open long	Spiral long
Spore shape	Oval	Barrel	Barrel	Oval
* Dimension	12x17mm	7x20mm	8x10mm	10x13mm

Magnification 10,000 X

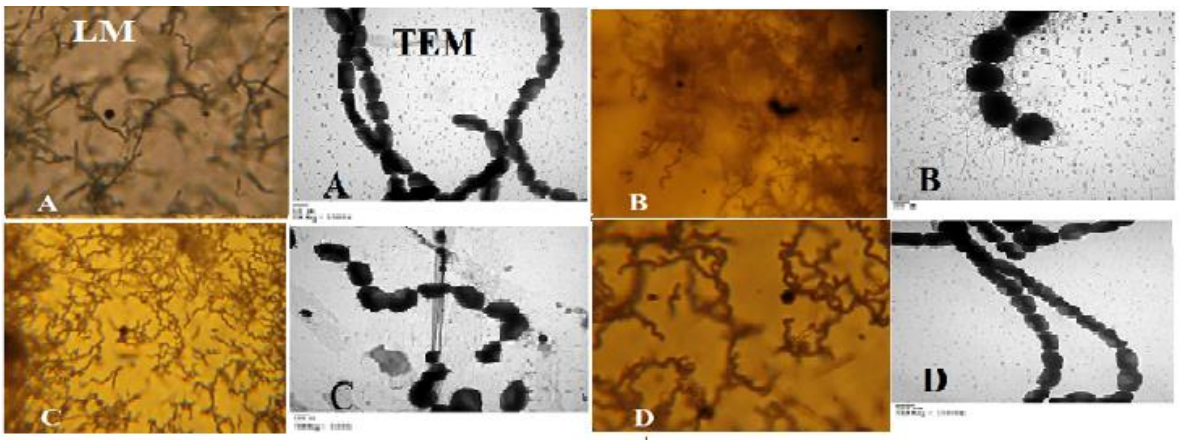

Fig. (2). Photo micrograph showing spore chains (LM, X 200) and spore surface (TEM, X 20.000) of four *Streptomyces scabies* isolates (A, B, C&D) by light and transmission electron microscopes.

***Streptomyces* Isolates Identification**:

The VITEK2 system was found to be valuable for the speed and accuracy of Gram-positive (GP) spore-forming. The four *Streptomyces spp.* isolates were Isolated and enumerated onto plates of starch nitrate agar medium. The Biochemical characteristics of VITIK2 system *Streptomyces scabies* isolates were confirmed with an excellent probability of 99%. Antibiotic sensitivity of *St.scabies* isolates showed different responses to antibiotic sensitivity ranging from sensitive, intermediate and resistant to by VITIK2 system.

Antibiosis:The four isolates showed antimicrobial potentialities against tested organisms except for St scabie 3 and St scabies 4 not showing against Ps. orugenosa. On the other hand, C. albicance and Aspergillus flavus appeared sensitive for isolates followed by followed by Ps. orugenosa (Table 3). The data showed that fungal isolates were more sensitive to five isolates than bacterial isolates. On the other hand, St. canarius showed higher antimicrobial potentialities against tested organisms due to an increase in relative inhibition.

Table 3: Antibiosis of *S scabies* isolates against microbial pathogenic.

Tested Organisms	*St scabies* 1		*St scabies* 2		*St scabie* 3		*St scabies* 4	
	DIZ (mm	R.I.	DIZ (mm)	R.I.	DIZ (mm	R.I.	DIZ (mm)	R.I.
C. albicance	32	42.5	48	68.4	35	46.4	28	41.4
Aspergillus flavus	24	40.2	39	52.5	30	39.5	30	42.8
B . ceriase	2.4	32.5	21	34.2	10	16.5	20	14.2
Ps. orugenosa	2.3	27.5	16	24.6	2	3.5	4	3.6

Rl = Relative inhibition DIZ = Diameter of inhibition zone (mm).

Potent Biodegradation of Gramonol:

In vitro: *Streptomyces scabies* population under study showed a great ability to degrade gramonol herbicide. They were different in their potential for degradation. The result concerning the amount of gramonol degradation is 11.66, 8.33. 7.50 and 12.50 mg with 21.20, 16.6, 15.0 and 25.0 % of *S. scabies* isolates St_1, St_2, St_3 and St_4 respectively (Table 4).

Table 4: The potent of *Streptomyces scabies* isolates on biodegradation of Gramonol herbicide.

St.. scabies isolates	Diameter cleaning Zone (cm)	Amount of gramonol degradation (ppm)	Percent of Degradation (%)
St scabies 1	3.50	11.66	21.20
St scabies 2	2.50	8.33	16.6
St scabies 3	2.25	7.50	15.0
St scabies 4	3.75	12.50	25.0

- Plate diameter 15 cm. - Gramonol amount in plate 5% (50 ppm/plate), Plate diameter 15 cm

Data concerning the degradation of gramonol in the population of Four *S. scabies* after 10, 20 and 30 days of soil treatment (table 5) showed that decrease gradually of gramonol amount in non-sterilized soil than in sterilized soil. The residual amounts of gramonol were 1050, 1600, 1550 and 750 ppm in sterilized soil after 30 days of soil treatment inoculated with four *St. scabies* respectively (table 5). On the other hand, the total count of *S. scabies* isolates decreased in the first days and then increased gradually under sterilized and non-sterilized soil (Table 4). The number of *S. scabies* isolates was 3.1; 2.2; 2.3 and 4.5 in sterilized soil while were 2.1, 7.1, 7.2 x 8.7 and $x10^5$ in non-sterilized soil at 30 days of four *St. scabies* respectively.

Table 5: Potent degradation of *Streptomyces scabies* isolates of gramonol in soil.

St. scabies isolates		Sterilized soil			Non-sterilized soil		
		Count	Gramonol residual		Count	Gramonol residual	
			(ppm)	(%)		(ppm)	(%)
Period soil inoculation (10 days)	*St scabies 1*	$6.0x10^4$	3700	74	$6.7x10^4$	2700	54
	St scabies 2	$4.1x10^4$	4000	80	$6.5x10^4$	3000	60
	St scabies 3	$3.7x10^4$	3750	75	$5.2x10^4$	3000	60
	St scabies 4	$5.7x10^4$	2600	52	$7.3x10^4$	2100	42
Period soil inoculation (20 days)	*St scabies 1*	$7.7x10^4$	2650	53	$7.1x10^4$	2000	400
	St scabies 2	$7.5x10^4$	2250	45	$7.2x10^4$	1750	35
	St scabies 3	$6.2x10^4$	2600	52	$6.5x10^4$	2050	41
	St scabies 4	$8.0x10^4$	1500	30	$8.5x10^4$	1000	20
Period soil inoculation (30 days)	*St scabies 1*	$3.1x10^5$	1050	21	$8.1x10^5$	900	18
	St scabies 2	$2.2x10^5$	1600	32	$7.1x10^5$	1050	21
	St scabies 3	$2.3x10^5$	1550	31	$7.2x10^5$	1250	25
	St scabies 4	$4.5x10^5$	750	15	$8.7x10^5$	500	10

Inject inoculum $5.2x10^4$ CFU *S. scabies* Gramonol is recommended at zero-time 5000 ppm.

Dehalogenase Activity:

Protein content was determined in *Streptomyces* isolate related to BSA standard protein (Table 6). *Streptomyces scabies*, isolate St_1 induced high protein content (2.15) followed by St_4 (1.95) and St_3 (1.85) and St_2, 1.75 mg/1g cells. The Dehalogenase activity was 285.2; 290.2; 275.6 and 235.7 unit/mg protein as well as, and the unit per gram growth were 499, 413.3; 425.3 and 357.5 for St_1, St_2, St_3 and St_4 isolates respectively. The *S. scabies* isolates revealed variation in dehalogenase rate activity. However, St_1 appeared higher rate of activity followed by St_4, St_2 and St_3 isolates (Table 6).

Table 6: Protein content and dehalogenase activity in *Streptomyces scabies* isolates.

S. scabies isolate	Protein content (mg/g growth)	Dehalogenase	
		Specific activity	(Units/g growth)
St. without gramonol	1.12	125.1	235.5
St *scabies* 1	2.15	285.2	499
St *scabies* 2	1.75	290.2	413.3
St *scabies* 3	1.85	275.6	425.3
St *scabies* 4	1.95	255.7	357.5

Specific activity (Unit/mg protein).

The Dehalogenase rate activity was varied among S. *scabies* isolates such as 4.00, 3.00, 2.00 and 3.50 for St_1, St_2, St_3 and St_4 isolates respectively at 7 min. (Table 7).

Table 7: Dehalogenase rate activity of *St. scabies* isolates:

Time min	St *scabies* $_1$	St *scabies* $_2$	St *scabies* $_3$	St *scabies* $_4$
0 time	1.25	0.95	0.75	1.15
1.0 min	1.75	1.49	0.95	1.50
2.0 min	2.50	1.75	1.25	2.25
3.0 min	2.75	2.10	1.50	2.50
4.8 min	3.25	2.25	1.75	3.15
5.0 min	4.00	3.00	2.00	3.50
6.0 min	4.00	3.00	2.00	3.50
7.0 min	4.00	3.00	2.00	3.50

Dehalogenase Activity Genetically:

The high purity and yield of DNA extract from *S. scabies* isolates which confirmed by UV spectrophotometer; 260/280 nm absorbance ratio were 1.75; 1.62;1.50 and 1.70 and DNA concentration was 31,28, 29 and 30 µg/0.5 growth of St_1, St_2, St_3 and St_4 isolates respectively.

The amplification of Dehalogenase gene of *S . scabies* isolates revealed variation among isolates, i.e. size and density of amplified DNA fragment with expected 500 bp (Fig. 3).

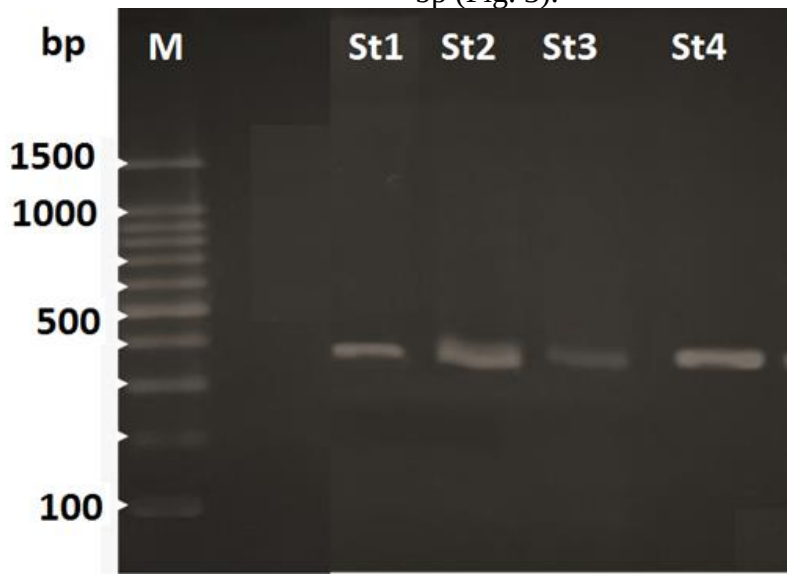

Fig. 3: Agarose gel 1.5% stained with ethidium bromide showing the PCR products of amplified Dehalogenase gene of 4 *S. scabies* isolates using specific primers. M. DNA leader 100 bp, St_1, St_2, and St_3 St_4: *St. scabies* isolates.

DISCUSSION

Occurrence and survival of potato scab pathogens (*Streptomyces* species) on tuber lesions, quick diagnosis based on a PCR-based assay (Lehtonen, *et al.*,2004). The herbicides are consumed annually in greater quantities for the control woods. The behavior of herbicides in the soil is regulated by herbicide properties, soil microbiology and climate conditions. The herbicide enters the plant seedling through the root and shoots. After being taken up, herbicide kill the plant seedling by interfering with photosynthesis, protein synthesis, enzyme systems, cell division, or other ways (Workman, *et al*., 1995). As well as during our chemical warfare against a multitude of noxious organisms in the soil. It is necessary to avoid the injury of these herbicides together with their various carrier diluents and solutes on the various beneficial soil microorganisms and their biological activities contributing to soil fertility.

Streptomyces scabies isolates showed potential variability to degrade gramonol herbicide *in vitro* and *in vivo*, which assimilation as a source of carbon and nitrogen. The analysis of gramonol residual by GLC at 10, and 20, 30 days post-inoculation showed the gradual decrease of gramonol residual was clearly in sterilized soil followed by non-sterilized soil. This difference due to microbial flora in soil may be antagonistic with Streptomyces scabies as well as had a role in the biodegradation of gramonol and in addition, this may be attributed to the concentration of soil (from drift occurrence of these herbicides).

The rate of herbicide application on the population density of soil microbes was studied by Roberts *et al.* (1993), who reported that *Pseudomonas* Sp. were important components of the population responsible for degradation of Limuron – Radio – respirometry studies, it showed that the culture mineralized Linuron completely. No intermediated degradation products were detected in the medium. These results are in agreement with those obtained by Tapp and Stotzky (1997), and Dahrog *et al.* (2006). It was found that herbicide concentration played a negligible role in the degradation behavior in the laboratory and they were bound on clay or silt-size particles or amended with the clay minerals.

The degradation of gramonol was detected in vitro with Streptomyces isolates but within different values among *Streptomyces scabies*. This obtained previous results are in agreement with those obtained by Hashish *et al*. (1990), Duhrog *et al*. (2006) and Azahar (2009), who studied the ability of soil microorganisms to dichlofunaid degrade the Linuron, Diuron, Ktozin and dichlofunoid. The actual degradation of the herbicide and fungicide by microorganisms is caused by the release of enzymes that break down them or the bacteria capable of significant biotransformation and reduced phenyl urea concentrations in liquid culture.

Investigation of Streptomyces scabies Causing Potato Scab by Various Detection Techniques, Its Pathogenicity and Determination of Host-Disease Resistance in Potato Germplasm (Ismail, *et al.*, 2020). Genomic selection for late blight and common scab resistance in tetraploid potato (Solanum tuberosum) G3 Gene Genomes (Enciso-Rodriguez, *et al.*,2018). The gramonol degradation by *Streptomyces scabies* isolates proceeds through the converted enzyme action that are specific for halogenated compound and enzymes that are involved in the metabolism of natural compounds. The former of the dehalogenase that catalyze hydrolysis of gramonol. The enzymes show a broad substrate range and one only produced by the isolates that utilize halogenated substrate and thus can be considered enzymes that are required by this specific strain of *Streptomyces scabies* during genetic adaptation to degradation of chlorinated hydrocarbons. The present study describes a further analysis of the data alkenes dehalogenase encoding gene dh1A. The absence of dehalogenase activities in the nature of *S. scabies* allowed the identification

of clones containing the Dehalogenase gene. Isolation of genes involved in methanol – Dehalogenase and chloro-acetaldhyde Dehalogenase activity was possible by screening for complementation of mutants lacking the Dehalogenase activities. In this way, harboring genes were identified and the genes were localized to different DNA segments (Bignell *et al.,*2010). The efficient expression of the halkinase Dehalogenase gene in other gram-positive bacteria is not suppressing in view of the fact that two regions with the consensus *E.coli* promoter sequence were present. Copy number probably also plays a role since expression levels were higher in *Xanthobacter autotropicus* G10 (p 120) than in the wild-type isolated G110. The *E. coli* consensus promoter sequence is known to stimulate transcription in *B . megatherium* (Dpyle *et al*., 1984 and Jeenes *et al*., 1986) and our data suggest that it might also do so in *Pseudomonas* sp. Genetic and physiological determinants of Streptomyces scabies were determined (Lerat et al.,2009) . In order to determine which of these sequences is the actual cause of the higher expression and whether the promoter can be used for the expression of other genes in *Pseudomonas* sp.; it will be necessary to identify the transcription short site of the gene and to study the expression of different gene linked to the promoter regions.

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ARABIC SUMMARY

المراقبة التحلل الحيوي لمبيدالجرامونول بعزلات الاستربتوميسس المسببة جرب درنات البطاطس

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4-قسم علوم الحياة والارض-المعهد العالى للمعلمين و التدريب نيجمينا –تشاد

تمت دراسة سلوك التحلل البيولوجي لمبيدات الآفات في مختلف النباتات الصالحة للأكل من قبل العديد من الباحثين . وخلصوا إلى أن المستويات الحالية لمبيدات الآفات في المياه السطحية والنباتات لا تشكل خطر السمية الحادة للإنسان على المدى القصير ، ولكن نتيجة لتراكمها في الجسم فإنها تسبب خطر التعرض على المدى الطويل . أظهرت الملاحظة البصرية بواسطة المجاهر الضوئية والإلكترونية لأربعة عزلات من *S. scabies* سميت (St1، St2، St3، St4) تميزًا في الجراثيم والكونيديا وكذلك تكوين صبغة منتشرة متغيرة في وسط تجريبي .وتم تعريفهم باستخدام نظام الفيتك . واحتمالية تحلل مبيدات الجرامونول فيما يتعلق بعزلات *S. scabies* المكتشفة بواسطة تفاعل البلمرة المتسلسل (PCR). أظهرت أربعة عزلات من S. الجرب قدرة محتملة مختلفة على تحلل الجرامونول في المختبر. وفي التربة. في الدورة 5 أيام حضانة و 35 يومًا بعد العلاج بمبيدات الأعشاب باحترام. من الواضح أن عزلة S. الجرب St4 كانت أكثر فاعلية في تحلل الجرامونول من العزلات الأخرى. تم الكشف عن جين Dehalogenase بواسطة PCR في جينوم DNA لـ 4 عزلات. وجدت فروقا بين العزلات مرتبطة بكثافة الشظايا وحجمها. يتم تحديد الجرامونول المتبقي بواسطة GLC بعد 10 و 20 و 30 يومًا. لوحظ زيادة معدل التحلل مع زيادة الوقت وكذلك لوحظ وجود كمية قليلة من بقايا الجرامونول في التربة غير المعقمة مقارنة بالتربة المعقمة فيما يتعلق بزيادة عدد الكائنات الحية الدقيقة الكلية. من ناحية أخرى لم يلاحظ تأثير الغرامونول على نمو عزلات *St. scabies*

الكلمات الافتتاحية : Streptomyces , Dehalogenase, Pesticides , PCR , GLC