Bioremediation of Oxamyl by some of Egyptian soil Bactria

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ABSTRACT:

Microbial degradation is the main process controlling the environmental dissipation of the nematicides. Oxamyl is an important Carbamate nematicide that is used for the control of nematodes in many economic crops in Egypt. Out of twelve bacterial isolates which were isolated from different Egyptian soils and showed high ability to grow and tolerate 40 ppm oxamyl as compared with other isolates, two bacterial isolates were found to be the most active decomposers of oxamyl in MSL medium, They were identified as *Brevibacillus gelatini* OX_1MZ265175.1 and *Brevundimonas diminuta* OX_2 MZ265176.1 based on morphological, biochemical characterization and 16S r DNA gene sequencing,. The environmental factors Affecting the bacterial growth and biodegradation of oxamyl at 40ppm, i.e.; temperature degrees, pH values and carbon and nitrogen sources were studied. The two identified strains were varied in their response to the different environmental factors. The determination of oxamyl degradation was performed using HPLC technique.

Keywords: Bioremediation; oxamyl; *Brevibacillus*; *Brevundimonas;* HPLC

INTRODUCTION

Bioremediation method uses biological agent like microorganisms to degrade the contaminants in the existence of sufficient nutrients and environmental conditions Kumar, *et al;* (2018). The degradation of xenobiotic compounds is an important indicator for healthy ecosystems; microorganisms can carry out pesticide degradation and can use the xenobiotic as a source of carbon, energy, and other nutrients to promote microbial growth (Durkin, 2003). oxamyl, for instance, has been classified by the U.S. Environmental Protection Agency (EPA) as Restricted Use Pesticide (RUP) due to its acute toxicity to humans, birds, and mammals Wilmington, (1990). This insecticide controls a broad spectrum of insects including nematodes. The oxamyl soil half-life is one to 5 weeks, with residual levels found for up to 6 to 12 months later. Microorganisms and pH were showed the two factors controlling the degradation of oxamyl in soils. It was well documented that the degradation of oxamyl is accelerated in neutral to alkaline soils Smelt *et al.,* (1983). In its extreme biodegradation, it could lead to the rapid dissipation of oxamyl in soils repeatedly treated with the Nematicide and eventually to loss of its biological efficacy (Smelt *et al.,* 1987). This phenomenon has been named enhanced microbial degradation and it has been attributed to the adaptation of a fraction of the soil microbial community to rapidly degrade oxamyl (Osborn *et al.,* 2010a). Similar studies reported that two aldicarb degrading bacterial strains were identified as *Stenotrophomonas maltophilia* (Karayilanoglu *et al.,* 2008) and *Methylosinus* sp. Osborn *et al*. (2010b) first reported the isolation of 27 oxamyl-degrading bacterial isolates were identified as *Aminobacter* and *Mesorhizobium* spp. However, no details on the metabolic pathway and the genes involved in the degradation of oxamyl were provided.

Bioremediation refers to any strategy were used to remove undesirable effects of pollutants from any environment using living organisms. Bacteria have been extensively used in bioremediation due to their fast growth, easy handling, and low cost. The *Micrococcus* spp. were so effective bioremediations that can degrade carbonyl, carbofuran, naphthalene, and many other aromatic compounds to be used as growth substances Doddamani and Ninnekar (2001). On the other hand, Tallur *et al*. (2008), showed that *Micrococcus* sp. was effectively used for degradation of cypermethrin, a pyrethroid pesticide.

The aim of this study was directed to isolate and characterize oxamyl-degrading soil bacteria, two isolates showed to be the most efficient in bioremediation of oxamyl nematicides as a sole source of carbon and nitrogen in mineral salt medium they were carried out to evaluate the role of environmental factors on the bacterial growth and bioremediation of oxamyl in mineral salt liquid medium.

MATERIALS AND METHODS

Soil samples

To isolate oxamyl degrading bacteria, tomato cultivating Egyptian soils namely, (Sadat and Nubaria) district were selected to achieved this study, these soils are well known repeatedly treated with oxamyl Nematicides. Soil samples were collected form the surface layer (0-20 cm), and kept to further studies

Nematicide used

Oxamyl (Vydate 24%) Nematicide which is used in the present study was obtained from local market of agricultural services, Tanta, Egypt. A Standard analytical grade solution of oxamyl was obtained from Central Agriculture pesticide laboratory, Agriculture Research center, Giza, Egypt.

Determination of oxamyl degradation by most efficient bacterial isolates

Five bacterial isolates which records as best isolates in growing and tolerate high concentration of oxamyl (40ppm), were individually inoculated in carbon and nitrogen free MSL medium (Liu, *et al* 2012): containing oxamyl 40ppm as a sole source of carbon and nitrogen. Incubation period was at 30°c for 7 days on rotary shaker at 150 rpm. After that, the oxamyl was extracted by its specific solvents and its residues were measured using High Performance Liquid chromatography (HPLC). Two bacterial isolates were recorded as the best in degrading oxamyl, and then selected for further studies in the present investigation.

Biochemical and Molecular properties of the two most efficient bacterial isolates;

Two most efficient isolates that performed highly degradation of oxamyl (OX1), and (OX2) were identified to the level species based on morphological, biochemical and 16S rDNA characteristics, and were found to be strains *Brevibacillus gelatini* OX_1MZ265175.1 and *Brevundimonas diminuta* OX_2 MZ265176.1 in respective order.

Environmental factors affecting bacterial growth and Bioremediation of oxamyl:

Effect of incubation periods

Concerning the two selected bacterial strains, each isolate was inoculated in MSL medium supplemented with 40 ppm of oxamyl. The cultures were incubated at different incubation periods, (3, 5, 7 and 9 days) on rotary shaker at (150 rpm).The percentage of degradation and the half-life of oxamyl was determined as described afterwards. Control flasks of equal volume of MSL medium and oxamyl without inoculation with bacterial isolates were applied. During the degradation, oxamyl samples were extracted periodically at 3, 5, 7 and 9 days intervals for estimating of oxamyl degradation.

Effect of different Temperature degrees:

The effect of temperature degrees on the bacterial growth and biodegradation of oxamyl was carried out using Erlenmeyer flask containing 100 ml MSL medium supplemented with 40 ppm of oxamyl, and then inoculated with each bacterial strain. Incubation periods were 25, 30, 35 and 40°C for 7 days. The percentage of oxamyl degradation was assessed using HPLC.

Effect of different pH values:

To evaluate the effect of pH values on the biodegradation of oxamyl, an experiment was performed using Erlenmeyer flask 250 ml containing 100 ml MSL medium and oxamyl was added as 40 ppm concentration, and1.0 ml of each bacterial strain. All treatments were adjusted at different pH values i.e. ;(6.0, 7.0, 8.0 and 9.0) then inoculated with and incubated at 30°C for 7 days. The percentage of oxamyl degradation was assessed using HPLC.

Effect of different Carbon sources:

This trial was designed to evaluate the bacterial growth and biodegradation of oxamyl under different Carbon sources, 100 ml of MSL containing 40 ppm of oxamyl amended with 10 g/L of various carbon sources namely, Glucose, Fructose, Sucrose and starch. Flasks were inculcated with 1.0 ml of each bacterial strain and incubated at 30°C for 7 days. Oxamyl degradation was assessed using HPLC.

Effect of different nitrogen sources:

To evaluate the effect of nitrogen sources and degradation of oxamyl, nitrogen free MSL medium was prepared by adding oxamyl (40ppm) and nitrogen sources i.e.; urea, ammonium nitrate, and ammonium sulphate were individually added. 1.0 ml of each culture strain was inoculated and incubated at 30°C for 7 days. Oxamyl degradation was assessed using HPLC

Analytical procedure:

Extraction:

A known volume of a MSL medium (80 ml), for each time interval, was transferred into

500 ml separator funnel and partitioned successively three times with 50 ml dichloromethane each and 40 ml of sodium chloride solution (20%). The combined extracts were filtered through a pad of cotton and anhydrous sodium sulfate then evaporated to dryness using a rotary evaporator at 30°C, then the residue was quantitatively transferred to standard glass stopper test tube with ethyl acetate, and the solvent was evaporated just to dryness and the residue became ready for chromatographic determination. The residue half-life value (RL50) was calculated using the equation of Moye *et al.,* (1987).

Chromatographic determination of oxamyl residues

The concentration of oxamyl were determined using an Agilent HPLC 1260 infinity series (Agilent technologies) equipped with a quaternary pump , a variable wavelength diode array detector (DAD), an auto sampler with an electric sample valve. The column was NucleosilC18 (30cm X 4.6 mm (i.d) X 5µm film thickness). The mobile phase was 60/40(v/v) mixture of HPLC grade acetonitrile/water. The wavelength was 220 nm and the mobile phase flow rate was 1 ml/min. the retention time of oxamyl under these conditions was 2.412 and the injection volume was 5 µL under these conditions.

Calculation of oxamyl biodegradation:

The oxamyl degradation was calculated by the following equation:

$$X\% = \frac{Cck - Cx}{Cck} \times 100$$

Where, X is oxamyl degradation; Cx is the concentration of oxamyl (mg l^{-1}) in the medium that has oxamyl degrading bacterial strain; CcK is the concentration of oxamyl (mg l^{-1}) in the medium that does not contain oxamyl degrading bacterial strain

RESULTS AND DISCUSSION

Screening for The most efficient isolates in degrading of oxamyl (40 ppm) as a sole source of carbon and nitrogen in MSL medium

Five efficient bacterial isolates (OX1), (OX2), B1, B2 and B3) were screened for the most efficient isolate in decomposing and utilizing oxamyl as sole sources of carbon and nitrogen in MSL medium. Results showed that (OX1) and (OX2) recorded the highest Figure in this respect Fig. (1) .The degradation percentages (%) of oxamyl were measured by HPLC. Results showed that the maximum percentage of degradation of oxamyl was recorded to reach the lowest value 90% for (OX1) then increased to 92% (OX2) respectively. Control (without inoculation) was reached to (22%). Incubation period was 7 days at 30±2C. The similar results were obtained by Mohamed. (2017) oxamyl was utilized by *Micrococcus luteus* in 48h in MSL medium supplemented with the Nematicide as a sole carbon source under shaking conditions. Osborn *et al:* (2010a) reported that oxamyl utilization by *Aminobacter* spp. and *Mesorhizobium sp.* on the other side, the different isolates of *Micrococcus* species were isolated from soils seem to be efficient bio remediators. They have been tested to degrade pyridine Gerald *et al.* (1986), cypermethrine (a pesticide) Tallur *et al. ;*(2015), carbaryl (insecticide), and other aromatic compounds. Carbaryl belongs to carbamate family of insecticide, like oxamyl, and a bacterium belongs to *Micrococcus* sp. which was isolated from a garden soil is capable to utilize carbaryl as a sole carbon source Doddamani and Ninnekar. (2001).

Identification of the most efficient bacterial isolates:

Wo bacterial isolates which showed highly effective in degrading and utilizing oxamyl, were identified on the basis of morphological and biochemical characters and 16S rDNA according to Belal and El-Nady, (2013). Results in (Table 2, 3) comprise several morphological and biochemical studies. The isolate (OX1) was found to be Gram-positive, motile, with Rod shape to oval colonies and Yellow to brown color and gave positive results for catalase, urease, citrate utilization and hydrolysis of starch, indole production and gelatin liquefaction ,while it showed negative results for oxidase,. The isolate (OX2) was found to be Gram-negative, motile, with Rod shaped, glossy, and convex and Whitish to cream color and gave positive results for, urease, indole production, catalase, citrate utilization and hydrolysis of starch, while it showed negative results for oxidase and gelatin liquefaction.

According to the 16S rDNA analysis (Boye *et al.,* 1999), the phylogenetic of two oxamyl degrading bacterial isolates namely, (OX1), and (OX2) was done Fig. (2and3). It can be clearly observed that Isolate (OX1) was included in the genus *Brevibacillus* and closely related to the species *gelatini*; it showed the highest sequence similarities with *Brevibacillus gelatini* OX1MZ265175.1 (95 %) as shown in

Figure (2). Concerning, the bacterial isolate (OX2). It was included in the genus *Brevundimonas* and closely related to the *diminuta* species; it showed the highest sequence similarities with *Brevundimonas diminuta* OX2 MZ265176.1 (99 %) as shown in Figure (3)

Environmental factors affecting bacterial growth and Bioremediation of oxamyl:

The efficiency of the biodegradation process of oxamyl can be enhanced by improving the conditions to be appropriate for the growth of the bacterial strains and bio-degradation. Degradation process; it takes into consideration many factors that may affect the growth of bacteria and their ability to boost the biodegradation in an optimal way; these factors including: Temperature, pH, incubation period, carbon and nitrogen sources. The biodegradation efficiency at different conditions was estimated based on the residual concentration of oxamyl after incubation with bacterial strains; the efficiency was calculated as a percentage based on the initial concentration. After incubation, percentage oxamyl degradation was measured using HPLC technique

Bioremediation of oxamyl under different incubation period

Results in Fig (4) recorded that different incubation period (3, 5, 7 and 9 days), showed caused variation in biodegradation capability of oxamyl. The *Br. gelatini* (OX1) recorded highest degradation percent % of oxamyl (40ppm) after incubation 9days (88%), the oxamyl active ingredients were recorded 4.88 ppm for 9 days followed by 14.4 ppm after 7 days of incubation, 25.56 after 5days and 26.32 ppm after 3 days . Similar results were recorded for *Br. diminuta* (OX2) where the highest degradation percent % of oxamyl (40ppm) after incubation 9days 90%, the oxamyl active ingredients was recorded 3.88 ppm for 9 days followed by 14.12 ppm after 7 days of incubation, 24.96 after 5days and 28.68 ppm after 3 days

In this study , Bioremediation percent of oxamyl 85-90 % was utilized by *Br. gelatini* (OX1) and *Br. diminuta* (OX2) in 7days using MSL medium supplemented with the oxamyl as a sole carbon source .these strains were be established the oxamyl and active ingredients comparison with degrading of present % un inoculation as control (30%) This result can be compared with those of Rousidou *et al*. (2016). They used four different newly isolated *Pseudomonas* strains to degrade oxamyl. Three of these strains completed the oxamyl degradation within 96h. Besides, Osborn *et al*. (2010a) reported oxamyl utilization by *Aminobacter* spp. and *Mesorhizobium* sp .

Bioremediation of oxamyl under different incubation temperature degrees;

Results abated in Fig (5) presents the degrading percentage of oxamyl at different incubation temperature degrees i.e., 25, 30, 35 and 40°C by the selected bacterial strains in liquid culture medium after 7 days of incubation and removing of active ingredients. The results showed that the incubation temperature at 25 ˚C was the best one for *Br. gelatini* (OX1), which the percentage of oxamyl degradation recorded its highest value(81.5 ,58,42 and 40 %) for 25, 30 ,35 and 40˚C respectively However the best incubation temperature degree was 40˚C for the oxamyl degradation by *Br. diminuta* (OX2), which reached (82 ,75 , 70 and 62 %) for 40, 35 ,30 and 25˚C respectively.

Bioremediation of the oxamyl under different pH values;

In the presents trial, the biodegradation of oxamyl at different pH values i.e., 6, 7, 8 and 9 was carried out by adding oxamyl (40 ppm) into conical flasks containing 100 ml MSL medium and inoculated with bacterial strains , the incubation temperature were 25 ˚C for *Br. gelatini* (OX1) and 40˚C for *Br. diminuta* (OX2) as the best one for each strains form the previous experiment .Results Fig (6) showed that, the strains (OX1) of *Br. gelatini* recorded the highest degradation percent at pH 7 which reached (73%) followed by pH 8 (59%), pH 9 (45%), and pH 6 (44%). As for *Br. diminuta*, (OX2) it was recorded its highest degradation percent at pH 7(79%) followed by pH 8 (67%), pH 9 (55%), and pH 6 (46%). The optimum pH for strain were 7.0 become give lowest restudies of oxamyl by HPLC which reached (10.7 and 8.14ppm) for (OX1) of *Br. gelatini, and* (OX2) *Br. diminuta*, respectively. The reason for reduction in pesticide degradation at lower or higher pH can be due to decreased activity of enzymes involved in pesticide degradation at acidic or basic conditions. In earlier studies Chen *et al*. (2012) and Jabeen *et al*. (2014) reported that neutral pH was optimum for microbial degradation of chlorpyrifos and cypermethrin. Also Cycon *et al*. (2009) reported that higher and lower pH significantly lowered the pesticides degradation by microbes. On the other side, Singh *et al*. (2004) found that that basic pH supports the microbial hydrolysis of CP.

Bacterial strains exhibited potential to degrade pesticides over a range of temperature (20-40°C), though the degradation potential was affected negatively at higher or lower temperature. The highest degradation was observed at 30°C and 35°C while a 30-40% decrease in pesticide degradation was noted at 25°C and 40°C. The pesticide degradation potential of microbes was further decreased to 60-75% at 20°C. Microbial growth and enzymatic activities occur usually at 25-35°C and temperature range for maximum pesticide dissipation is 25-40°C (Chowdhury *et al*., 2008). Various pesticide degradation studies using microbes have demonstrated that 30-35°C is the optimum temperature for microbial degradation activity (Li *et al*., 2010; Zhang *et al*., 2011a; Chen *et al*., 2012; Singh *et al*., 2012; Wang *et al*., 2012).

Bioremediation of oxamyl under different carbon and nitrogen sources

This trial was designated to study the effect of carbon and nitrogen sources on the biodegradation of oxamyl by with the different carbon sources i.e. glucose, fructose, sucrose and starch, to a final concentration of 0.01% (w/v), as well as different source of nitrogen namely ammonium chloride, urea, sodium nitrate and ammonium sulphate to a final concentration of 0.05% (w/v) in MS medium containing oxamyl (40 ppm) and incubated at 25 and 40 C for *Br. gelatini* (OX1) and *Br. diminuta* (OX2) receptivity after 7days. The Results in Fig (7) showed that degradation % of oxamyl by the two bacterial strains as affected by the carbon sources. Glucose was found to be the best one for two tested strains, where *Br. gelatini* (OX1) and *Br. diminuta* (OX2), recorded the degradation% 90 and 86% respectively. The least degradation %was 60% for *Br. diminuta* (OX2) in the present of starch, and was 66% for *Br. gelatini* (OX1) when sucrose used.

Regarding the nitrogen sources, Fig (8) degradation % of oxamyl by the two bacterial strains as affected by the nitrogen sources was studied. As for urea, it was found to be the best nitrogen sources for the degradation % of oxamyl by the *Br. gelatini* (OX1), It was (84%). While *Br. diminuta* (OX2) recorded (89%). It was observed that the least degradation % of oxamyl recorded (62% and67%). In respective order for *Br. gelatini* (OX1) and *Br. diminuta* (OX2) when ammonium chloride added as a sole source of nitrogen in MSL medium.

Addition of glucose as a supplementary carbon source significantly enhanced oxamyl degradation by most of strains by 86-90 %. The glucose as immediate energy sources enhanced cell growth which in turn increased oxamyl degradation. Similar enhanced degradation of pesticide by adding carbon source has been reported in earlier studies (Cycon *et al*., 2009; Pino and Peñuela, 2011). It can also be assumed that organisms not only metabolized oxamyl but also co-metabolized these in the presence of other C source as observed before for degradation of pesticides (Chen *et al*., 2012). In contrast, few reports pointed out that additional Carbon source (glucose) reduced degradation activity of microbes, possibly because of utilization of glucose as Carbon sources in place of pesticides (Karpouzas *et al*., 2005; Abo-Amer, 2012). As addition of Carbon source suppressed chlorpyrifos degradation by *Enterobacter sp*. for first three days and cyparmthren degradation started after depletion of Carbon source (Singh et al., 2004).

On the other Hand, the use of nitrogen sources in medium might enhance the biodegradation of pesticides. Similar results were obtained by Gangireddygari et al., (2017) to detect the influence of nitrogen sources urea , ammonium chloride, ammonium sulfate, or yeast extract to a final concentration of 0.01% (w/v)) on the biodegradation of quinalphosby *Bacillus thuringiensis*, *Bacillus pumilus* and two strains of *Pseudomonas putida*, supplementation of nitrogen, except for the yeast extract, were improved degradation of quinalphos.

On the other hand, availability of essential nutrients such as carbon, nitrogen, phosphorus and oxygen may also limit the rate at which bacteria degrade pesticides. Recently a *Pseudomonas* able to mineralize atrazine has been enriched from soil; this organism used atrazine as the sole source of nitrogen with sodium citrate as the carbon source (Mandelbaum *et al*., 1995); the use of additional nitrogen source in the medium may cause negative effects on the biodegradation of atrazine in this case (Aislabie and Lloyd-Jones, 1995).

CONCLUSIONS

In the present study, two effective bacterial isolates,i.e, OX1 and OX2 were found to be the most efficient isolate in decomposing and utilizing oxamyl (40ppm) as carbon and energy source .They were identified based on morphological, biochemical and 16s rDNA characterization to be strains of *Brevibacillus gelatini* OX1 and *Brevundimonas diminuta* OX2 .The two strains were carried out to evaluate the effect of different environmental factor on

utilization and degradation of oxamyl. Results showed that the two strains were varied in their response to the environmental factors, and the two bacterial strains could remove up to 85-90 % oxamyl from the medium; hence they may be suggested for bioremediation of oxamyl contaminated soil, water and industrial effluents.

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Table 1: Morphological characteristics of colonies of the most efficient oxamyl degrading bacterial isolates

Bacterial isolates character	Isolate (OX1)	Isolate (OX2)
Gram Reaction	Positive	Negative
Colony shape	Rod shape to oval colonies	Rod shaped, glossy, and convex
Color	Yellow to brown	Whitish to cream
Margin	Entire	Entire
Elevation	Raised	Raised
opacity	Turbid	Transparent
Motility	Motile	Motile
Spore formation	spore former	Non Spore former

Table 2: Biochemical characteristics of the most efficient oxamyl degrading bacterial isolates

Bacterial isolates Properties	Isolate (OX1)	Isolate (OX2)
Oxidase	Negative	Negative
Catalase test	Positive	Positive
Urease	Positive	Positive
Citrate Utilization	Positive	Positive
Indole production	Positive	Positive
Gelatin liquefaction	Positive	Negative
Hydrolysis of starch	Positive	Positive
hydrolysis of cellulose	Positive	Positive
Growth in NaCl		
2%	+++	++
5%	+++	++++
7%	+++	++
10%	++	++
Growth at temperature		
25	++	++
30	++++	++++
35	+++	+++
40	++	++
45	++	+

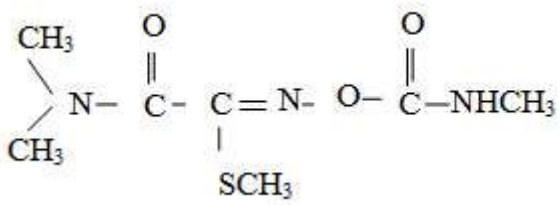

Figure: Chemical structure of oxamyl (Methyl, N, N- dimethyl -N-[(methyl carbamoyl) oxy] -1-thiooxamimidate)]

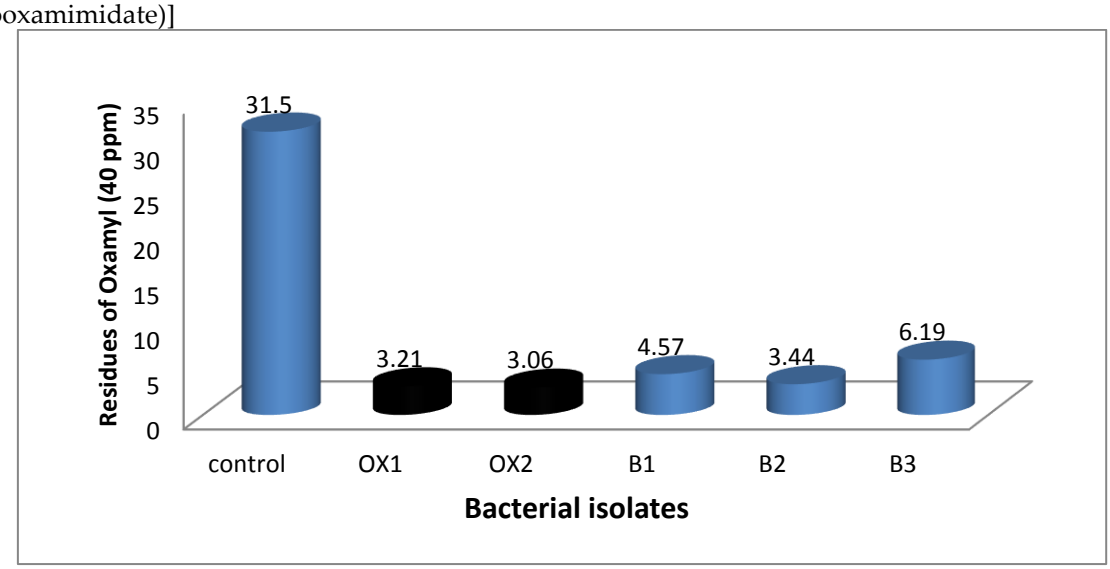

Figure 1: Residues of oxamyl (40 ppm) in MSL medium as a sole source of C and N after inoculation of bacterial isolate for 7days

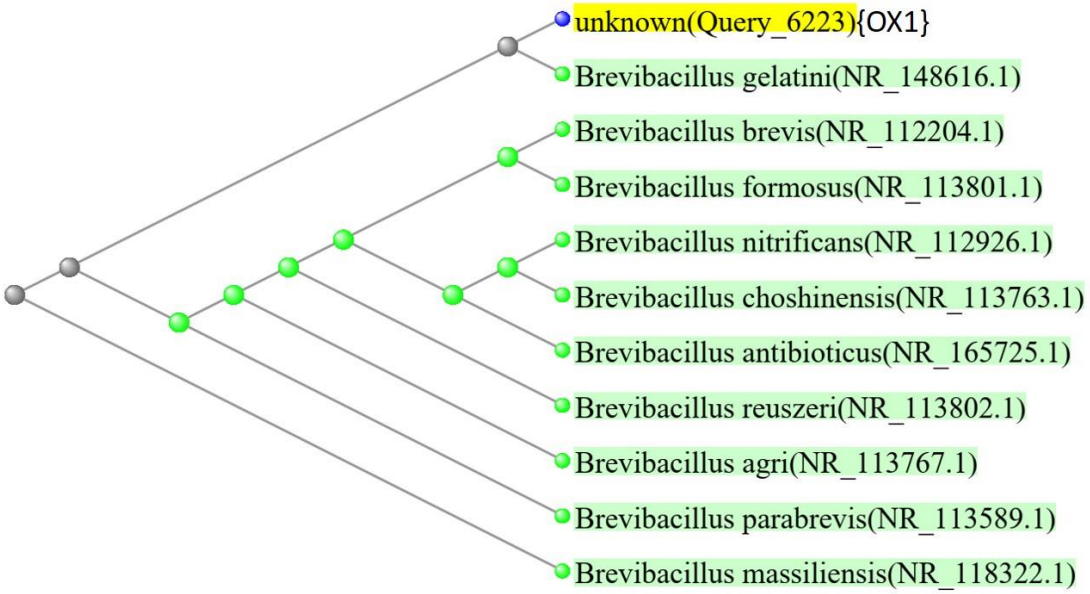

Figure 2: Phylogenetic tree of the strain (OX1) in contrast to the most closely related bacterial strain in the NCBI database (*Brevibacillus gelatini* OX1MZ265175.1).

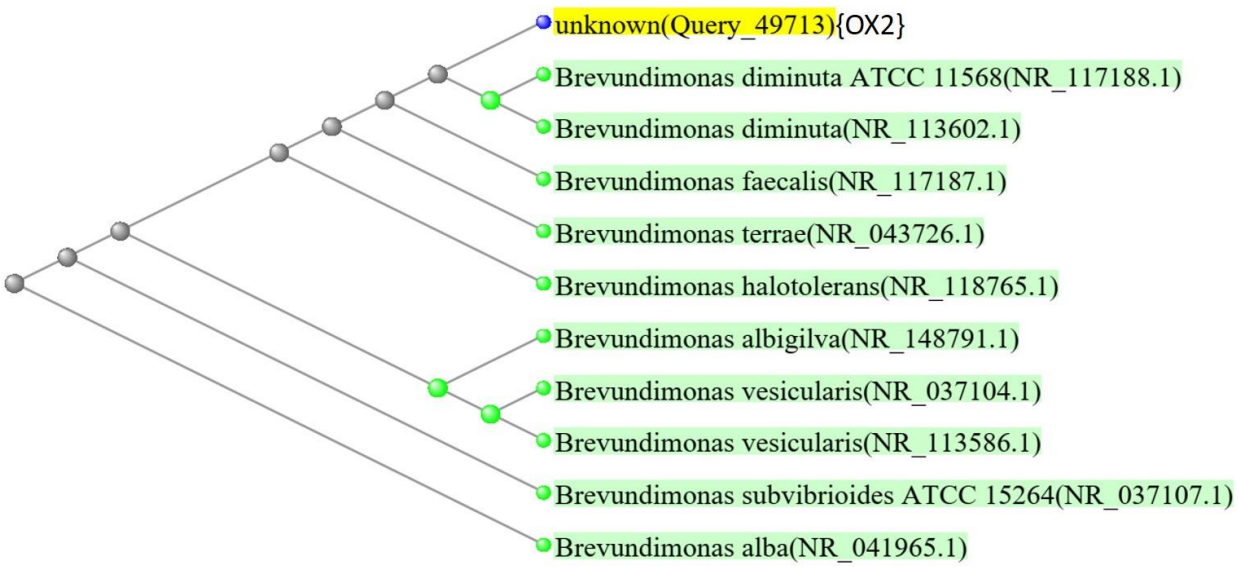

Figure 3*:* Phylogenetic tree of the strain (OX2) in contrast to the most closely related bacterial strain in the NCBI database *(Brevundimonas diminuta* OX2MZ265176.1*).*

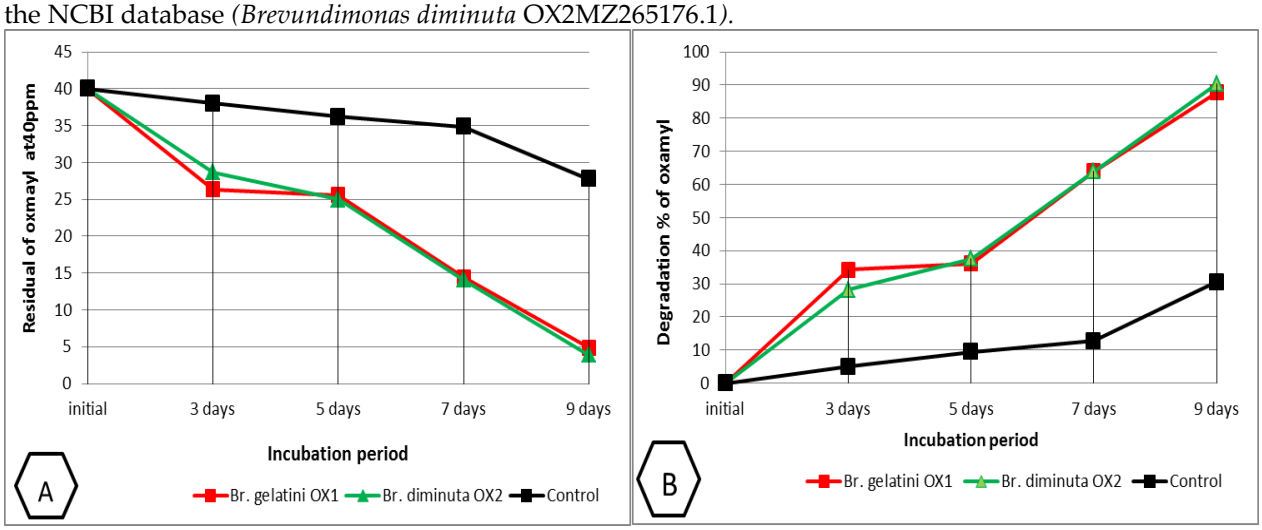

Figure 4: Bioremediation of oxamyl under different incubation period

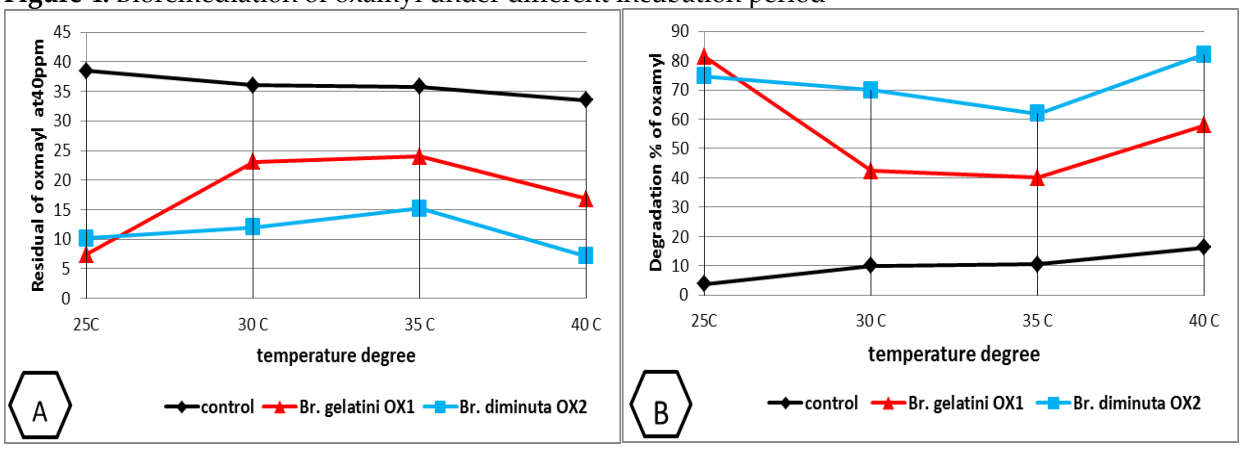

Figure 5: Biodegradation of oxamyl under different incubation temperature degrees.

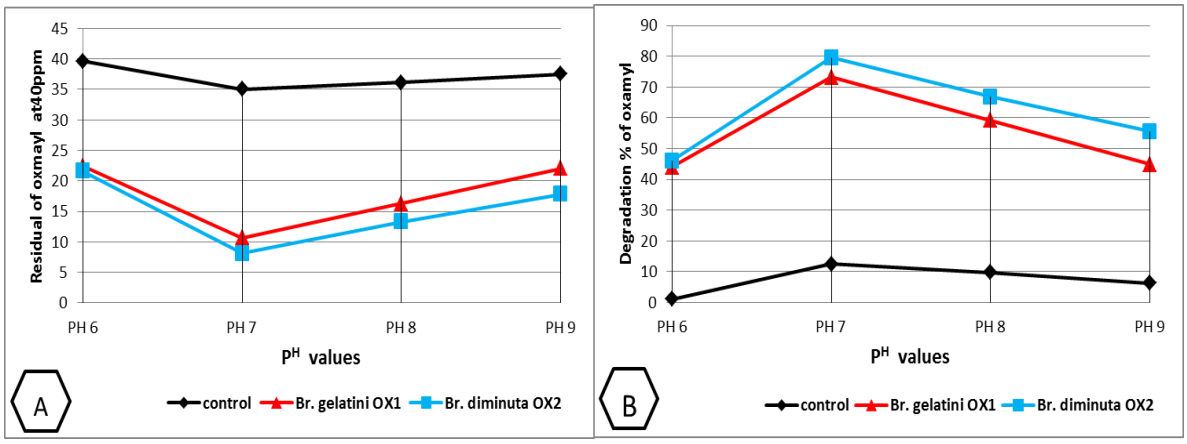

Figure 6: Bioremediation of the oxamyl under different pH values

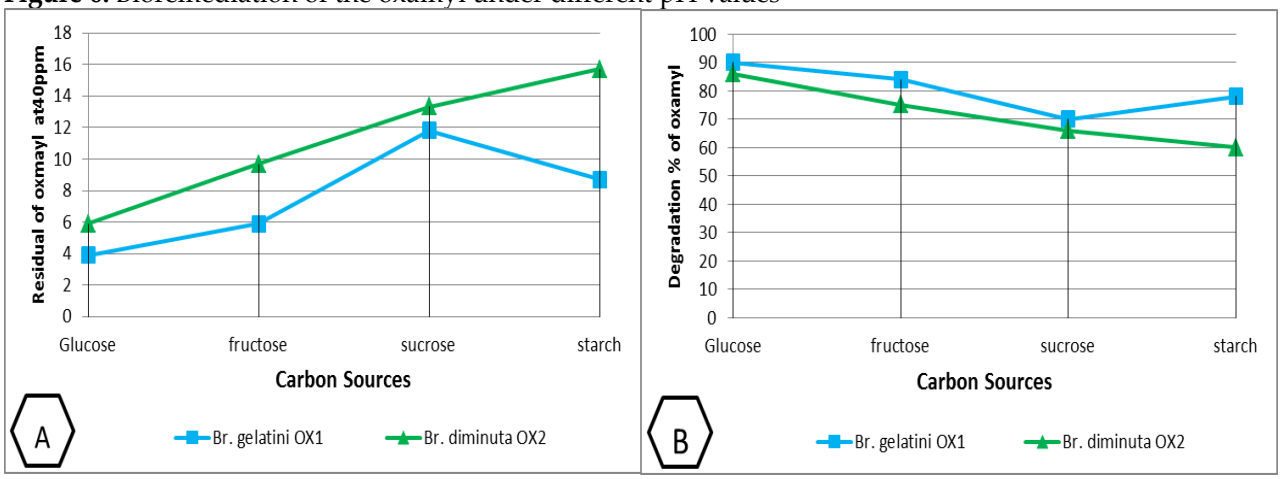

Figure 7: Bioremediation of oxamyl under different carbon sources.

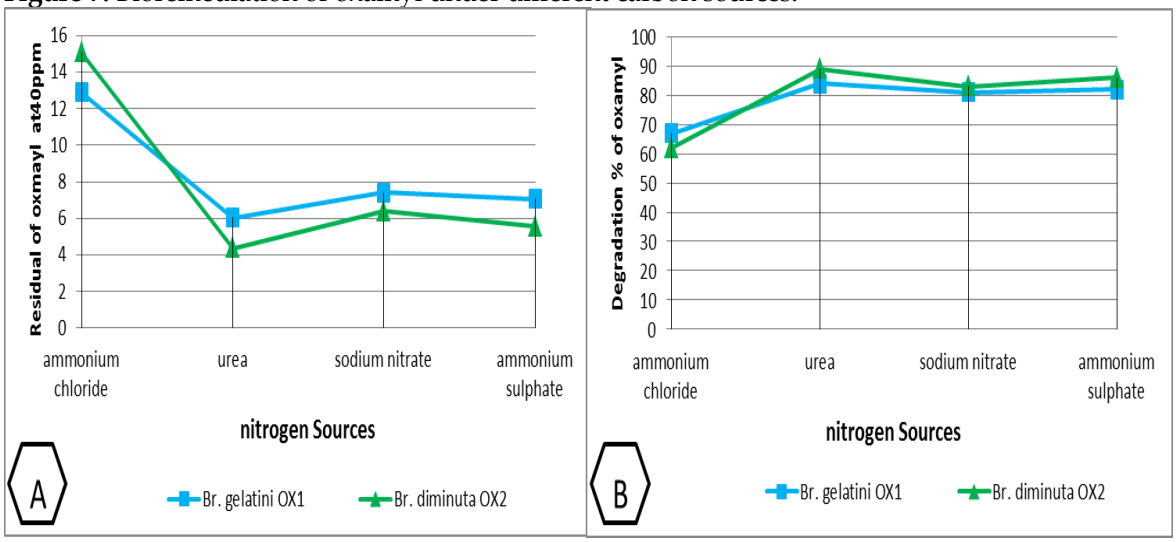

Figure 8: Bioremediation of oxamyl under different nitrogen sources

المعالجة الحيوية للأوكساميل بواسطة بعض بكتريا التربة المصرية

عبدالله بديع الديب [1,*] , هند عبداللاه محمود [2] , عادل عبد الفتاح شكري [1] , عبدالعزيز فتحى الحماحمى [1]

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الملخص العربى

من بين اثني عشرة عزلة معزولة من تربة مزروعة طماطم من منطقة (السادات والنوبارية) ، تم الحصول على سلالتين من البكتيريا و تم تعريفها على أساس خصائصها المورفولوجية والبيوكيميائية والتسلسل الجين 16Sr DNA تبين ان إحداهما *Brevibacillus gelatini* OX1 MZ265175.1 والأخرى *Brevundimonas diminuta* OX2MZ265176.1, حيث أظهرت هذة العزلات كفاءة في التحلل البيولوجي واختفاء المادة الفعالة فى المبيد و إستخدام الأوكساميل كمصدر وحيد للكربون في بيئة (MSL). كما أظهرت النتائج دراسة تاثيرالعوامل البيئية على نمو البكتيريا والتحلل الحيوي للاوكساميل . حيث إختلفت العزلات في استجابتها للعوامل البيئية المختلفة. و تم تحقيق أقصى قدرة على تحلل الأوكساميل عند 40 جزء في المليون خلال 7 أيام عند قيمة pH= 7 ودرجة حرارة 25 و 40 درجة مئوية للعزلات *Br. gelatini* و *Br. diminuta* على التوالي.وكان الجلوكوز هو أفضل مصادر الكربون بينما كانت اليوريا أفضل مصادر النتروجين.

الكلمات الاسترشادية : المعالجة الحيوية – الأوكساميل – بكتريا *Brevibacillus* و *Brevundimonas*