

DISSIPATION OF PENDIMETHALIN BY *Bacillus megaterium*

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ABSTRACT

One strain of microorganisms was isolated from soil previously treated with pendimethalin using enrichment technique and identified as *Bacillus megaterium* (E22). The effect of pH and temperature on the growth ability of the tested strain was investigated. The results showed that the optimum pH and temperature for the growth of pendimethalin degrading strain were 7 and 30 °C, respectively. Some concentration of pendimethalin was removed by *B. megaterium* remove from mineral liquid medium with half-life of 5.6 days. Pendimethalin half-life was 53.4 days in untreated mineral liquid medium as control. Also, there is no toxicity of pendimethalin detected on *Rhizobium leguminosarum* biovar viciae as test microorganism in the supernatant after 28 day of treatment with *B. megaterium*. The results suggest that bioremediation by *B. megaterium* isolate was considered to be effective method for detoxification of pendimethalin herbicide in aqueous media.

Keyword: Pendimethalin, Soil, Bioremediation, phytotoxicity, seedling mortality.

INTRODUCTION

Pendimethalin, N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine, had empirical formula $C_{13}H_{19}N_3O_4$, a selective preemergent herbicide in dinitroaniline group was used extensively for weed control in cotton, rice, soybean and tobacco (Smith *et al.*, 1995). Pendimethalin acts by inhibiting the steps in plant cell division responsible for chromosome separation and cell wall formation. It is used before crop emergence or planting (Appleby and Valverde, 1988). The inhibition of root and shoot growth results in stunting of aerial plant Portions (Parka and Soper, 1977). Studies in terrestrial ecosystems showed that 10–20% of the herbicide vaporizes within the first week or two weeks after application (Strandberg and Scott-Fordsmand(2004). Care should be taken to minimize excessive pendimethalin applications to the soil in order to minimize possible injury to sensitive rotation crops. It is important to develop the methodology to prevent pesticide contamination from. Microorganisms can use a variety of xenobiotic compounds including pesticides for their growth, mineralize and detoxify them (Belal *e tal.*, 2008). Bioremediation is an accepted technology for accelerating the rate of cleanup of contaminated water and soil. Soil microorganisms that are repeatedly exposed to pesticides may develop new capabilities to degrade such chemicals (Vidali, 2001). There are some reports on the degradation of pendimethalin by microorganisms comprising *Azotobacter chroococcum*, *A. vinelandii* and *Bacillus circulans* (Saha *et al.*, 1991; Singh and Kulashrestha, 1991; Kole *et al.*, 1994; Megadi *et al.*, 2010). The success of bioremediation depends not only on the high degradation ability but also on the stability of active microorganisms under varied conditions, such as changes in pH and

temperature (Pattanasupong *et al.*, 2004). Therefore, it is necessary to investigate the effects of various environmental factors on the growth ability of the tested microorganisms (Pattanasupong *et al.*, 2004). However, after remediation toxicity assessments are needed. Firstly, is providing valuable and complementary information to compound analysis. Secondly, the major advantage of toxicity tests is the direct assessment of the potential hazard to the environmental system by both original pollutants and its metabolites (Tiainen *et al.*, 2002). Therefore, this study attempted to isolate and identify efficient bacterial strain for bioremediation of pendimethalin in aquatic. In addition to confirm the complete detoxification of pendimethalin by measuring the toxicity of the aqueous medium in the presence of bacterial strain against sensitive target such as *Rhizobium leguminosarum* biovar *viciae*.

MATERIALS AND METHODS

Chemicals

Pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) standard was obtained from Ehrenstorfer (Germany). All other chemicals were of analytical grade.

Microbial degradation of the pendimethalin :

Media

M9-Minimal medium as mineral salt liquid (MSL) and Luria Bertani (LB) a complete medium were used through this study as described by Sambrook *et al.* (1989).

Isolation by enrichment culture

Enrichment cultures of microorganisms capable of degrading pendimethalin were established from soil that previously treated with pendimethalin. Samples of soils were collected from Kafr El-Sheikh and Elbeheira, Governorates, Egypt. Ten grams soil were suspended in 90 ml sterilized mineral salt medium in 500-ml bottle containing (100 µg/ml) of pendimethalin as a sole source of carbon, then incubated at 30 °C and 150 r/min for 28 days. Thereafter, 10 ml of the cultures were transferred into fresh 90 ml MSL medium containing the same concentration of pendimethalin. This procedure was repeated four times. Series dilutions were prepared after the final time from enrichment culture in a glass tube containing 9 ml MSL medium up to $1:10^{-6}$ and then 100 µl of it was speeded on plates of MSL medium + pendimethalin (100 µg/ml) using Drigalsky triangle. The plates were sealed in polyethylene bags, then incubated at 30 °C for 7 days and monitored for appearance of colonies. Single colony growing on each plate was isolated by picking the colony using sterile inoculating needle and was further purified by the standard spatial streaking on complex agar media for bacterial isolate. The isolated colonies were then tested for their ability to grow in MSL medium containing (100 µg/ml) of pendimethalin. The number of cells of each strain was determined by plating appropriate dilutions of liquid medium onto mineral salt agar medium containing pendimethalin. Bacterial populations were estimated by counting the number of colonies on plates (Belal and El-Nady 2013).

Identification

The efficient selected pendimethalin degrading bacterial isolate was identified depending morphological and physiological characteristics as described by to Parry *et al.*, (1983).

Effect of temperature and pH on the growth of the tested strain:

To determine the effect of temperature and pH on the growth of tested strain, a 30 ml MSL medium supplemented with 100 µg/ml of pendimethalin as a sole source of carbon for bacterial strain. MSL medium was inoculated by one ml from bacterial cell suspension at 10^7 cfu/ ml. To determine the optimum pH, experiments were carried out at pH 6, 6.5, 7, 7.5 and 8. Cultures were incubated on a rotary shaker at 30 °C and 150 r/ min for 7 days. To determine the effect of temperature, MSL medium with pH of 7 was incubated at 20, 25, 30, 35 and 40 °C under 150 r/min. Cells number of the bacterial strain were determined by plating appropriate dilutions of liquid medium onto mineral salt agar medium containing pendimethalin.

Biodegradation of pendimethalin by *Bacillus megaterium* (E22) in liquid medium:

Bacillus megaterium (E22) was cultured onto MSL medium + pendimethalin for 7 days and then the growing colonies were washed with 3 ml sterilized MSL medium. The bacterial cell suspension (10^7 cfu/ml) was then used to inoculate 100 ml MSL medium containing (100 µg/ml) of pendimethalin. The cultures were incubated at 30 °C and 150 r/ min for 0, 7, 14, 21 and 28 days. The percentage of degradation and the half-live of pendimethalin were determined as described afterward. Control flasks of equal volume of liquid mineral medium and pendimethalin without any microbial population were run in parallel at all intervals to assess a biotic loss.

Analytical procedure :

Extraction and determination of pendimethalin residues was carried out by the described method by Jazwa *et al.* (2009) at Central Agric. Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. The analysis of the samples was performed using a Hewlett Packard 5890A gas chromatograph, equipped with nitrogen – phosphorus detector (GC-NPD). The column used in this study was an HP fused – silica capillary column coated with cross-linked methyl silicone (length 25 m, ID 0.31, film thickness 0.52 µm). Nitrogen was used as both the carrier and make-up gas at a flow rate of 30 ml/min. Hydrogen was used at a flow rate of 3.5 ml/min. and air at 120 ml/min. The oven temperature was programmed as follows: initial temperature 150 °C (1 min.), rate of 10 °C/min. and final temperature 250 °C. Recovery studies were carried out regularly by spiking analytical samples with stock solution of pendimethalin standard.

Toxicity test:

The toxicity bioassay of pendimethalin was performed on the aqueous solutions after 28 days after treatment with the tested microbial isolates. *Rhizobium leguminosarum* biovar *viciae*, as a test microorganism to pendimethalin was used as the test organism. The toxicity was determined as percentage of inhibition in the growth of tested bacteria comparing to control treatment. Yeast extract manitol agar medium was poured into Petri dishes (9 cm in diameter, 15 ml /dish), after solidification, wells were punched

in each plate. The plates were inoculated by spreading 100 µl (10^8 cfu/ ml) from growth suspension of *Rhizobium leguminosarum* biovar *viciae* on Yeast extract manitol agar plates. After that, 50 µl from the supernatant was put in punched holes (0.5mm in diameter) in Yeast extract manitol agar plates, where the culture broth was obtained by culturing of the pendimethalin degrading isolate with pendimethalin in MSL medium after 28 days. The culture broth was filtrated through a sterile membrane filter (0.2 µm). On the other hand, 50 µl from sterilized liquid medium was put in punched holes which were used as control treatments. Experiments were made in three replicates. Plates were incubates at 28°C. Diameter of inhibition zone (mm) surrounding each hole was recorded after 72hrs.

RESULTS AND DISCUSSION

Isolation of the pendimethalin-degrading isolates:

The pre-treated soil samples with pendimethalin were used to isolate the pendimethalin-degrading microorganisms in the present study. By using enrichment techniques,. A preliminary classification based on the morphology of the isolates revealed that, the pendimethalin-degrading microorganisms belongs to the group of bacteria. The isolate was Gram positive, motile, spore forming rod shaped bacterium. This bacterial strain (E22) was identified according to morphological, physiological chacterstics as *Bacillus megaterium*.

Our results are in agreement with previous finding reported by (Kopytko *et al.*, 2002; Karpouzas *et al.*, 2005; Belal *et al.*, 2008; Megadi *et al.*, 2010). It was found that enrichment culture technique led to the isolation of two bacterial strains, which were able to degrade different pesticides rapidly in liquid cultures. Chaudhry and Ali, 1988 reported that, the application of pendimethalin promotes the evolution of microorganisms that are capable of degrading this xenobiotic compound in the soil.

Also, Chaudhry and Ali, (1988) reported that, actinomycetes have considerable potential for the biotransformation and biodegradation of pesticides. On the other hand members of this group were gram-positive bacteria and have been found to degrade pesticides with widely different chemical structures including organochlorines, striazines, triazinones, carbamates, organophosphates, organophosphonate, acetanilides, sulfonylureas and herbicide,metolachlor (Krause *et al.*, 1985; De Schrijver and De-Mot, 1999).

Effect of environmental factors on the growth of *Bacillus megaterium* (E22) :

Effect of pH

Normally, the pH and temperature influence the growth of microorganisms and hence, these factors will influence also the degradation process of the pesticides. Karpouzas and Walker (2000) reported that the degradation of ethoprophos by *P. putida* strains epl and II affected by pH and temperature. Hong *et al.* (2007) found that various factors including pH and temperature affected degradation of fenitrothion-contaminated soil using *Burkholderia* sp. FDS-1. Belal *et al.* (2008) found also that the pH and

temperature affected cadusafos, carbofuran and cymoxanil degrading microorganisms comprising fungi and bacteria. The influence of pH on biomass yield by the tested isolates is shown in Fig. 1.

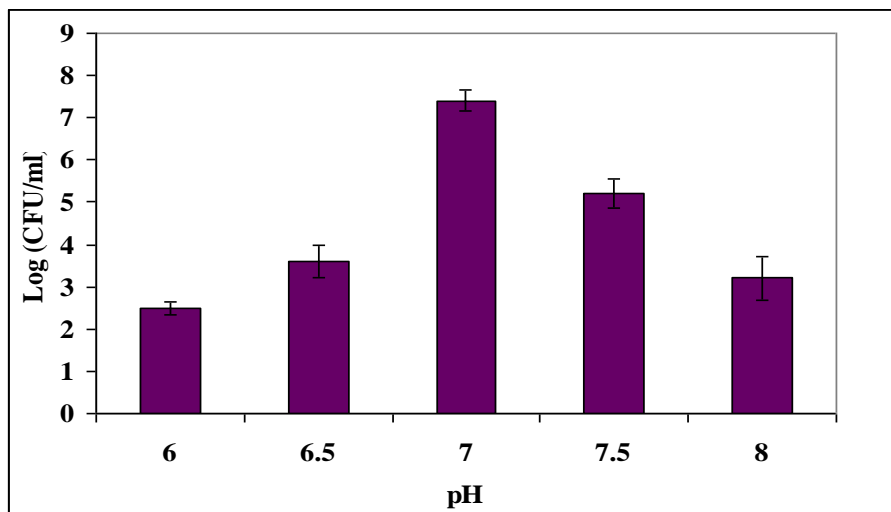


Figure 1: Effect of pH on the growth ability of *Bacillus megaterium* (E22).

Generally, the optimum pH was 7 for either bacterial or fungal isolates. Since, the maximum intracellular protein content for *Bacillus megaterium* (E22) were recorded at pH 7 (Fig. 1). It is known that the most of bacterial isolates prefer the neutral pH. However, the tested bacterial strain in this study can grow at range of pH from 6 to 8.

Ross and Marco, (1978) reported previously that metalaxyl acid (N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine) resulting from the hydrolysis of the methyl ester group of pendimethalin which determined as the major metabolite in field soils at potato harvest and may this allow to effect degrading of metalaxyl.

Effect of temperature

The effect of different temperatures on the growth of *P. putida* (E22) is shown in Fig. 2. The temperature of 30 °C appears to be the optimum degree for growth of *Bacillus megaterium* (E22). Moreover, the tested microbial isolates exhibited growth at 40 °C. of *Bacillus megaterium* (E22) was used for further studies under the optimum growth conditions to evaluate their degradation potential for pendimethalin at different incubation times (0, 7, 14, 21, 28 days).

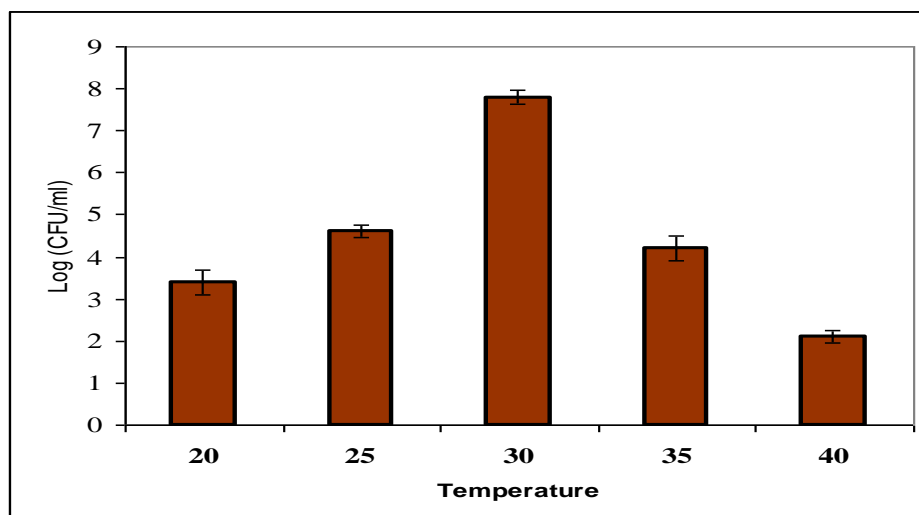


Figure 2: Effect of temperature on the growth ability of *Bacillus megaterium* (E22).

Biodegradation of pendimethalin by *P. putida* (E15) in aquatic system

The ability of *Bacillus megaterium* (E22) to degrade pendimethalin was illustrated in Fig. 3. The results indicate that *Bacillus megaterium* (E22) was most efficient strain in pendimethalin degradation with a half-life of 5.6 days. One hundred percent of pendimethalin initial concentration was degraded within 4 weeks by *P. putida* (E15). Pendimethalin half-life was 53.4 days in untreated liquid medium as control treatment.

The growth response of pendimethalin *Bacillus megaterium* (E22) was increased gradually with the pendimethalin degradation rate increasing as shown in Fig. 3.

This is suggesting that different microbial types, which may be using different enzymes, have different degradation preferences. On the other hand, pendimethalin degradation percentage reached to 17% at the end of incubation time in control or non-inoculated samples. This implies that the quote of pendimethalin decay due to temperature effect and volatilization (Strandberg and Scott-Fordsmand, 2004). Many authors reported earlier that *Pseudomonas* has considerable potential for the biotransformation and biodegradation of pesticides. Members of this group are gram-negative bacteria and have been found to degrade pesticides with widely different chemical structures (Spain and Nishino, 1987; Kyria *et al.*, 1997). The degradation of some pesticides may be attributed to the secretion of enzymes from either tested bacterial or fungal strains which are capable of degrading of pesticides (Bollag and Liu, 1990).

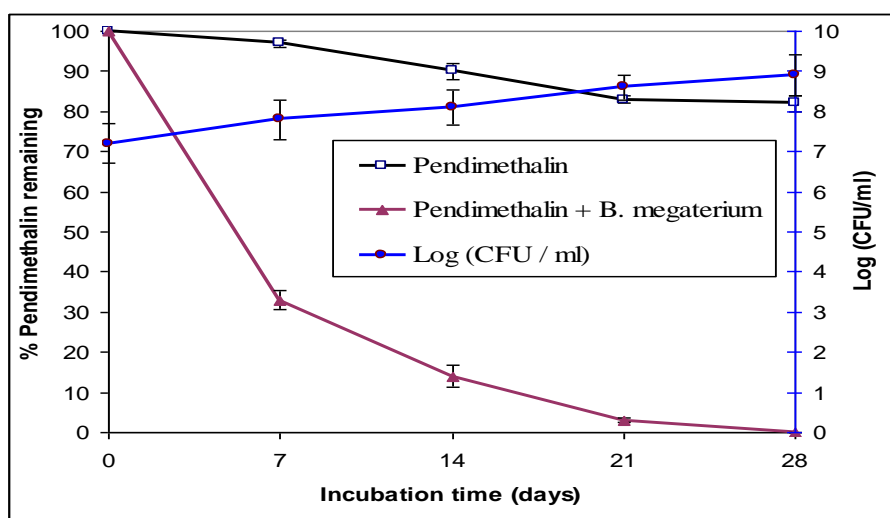


Figure 3: Biodegradation of pendimethalin by *Bacillus megaterium* (E22) in aquatic system.

With regard to biological metabolization, in vitro degradation of pendimethalin has been demonstrated by numerous authors. For instance, Kole *et al.*, (1994) observed that 45% and 55% metabolism of pendimethalin caused by *Azotobacter chroococcum* after 10 and 20 days of incubation, respectively. *Azotobacter vinelandii* was isolated from a pendimethalin-treated barley rhizosphere. *A. vinelandii* utilized pendimethalin as the sole source of carbon to fix N_2 (Saha *et al.*, 1991; Singh and Kulashrestha, 1991). Pendimethalin was degraded by oxidative N-dealkylation to yield 3,4-dimethyl-2,6-dinitroaniline and pentane. However, 6-aminopenimethalin and 3,4-dimethyl-2,6 dinitroaniline were not further metabolized because they neither supported growth of organism nor stimulated oxygen uptake. But the pentane, released by oxidative N-dealkylation of pendimethalin, was utilized as the sole source of carbon and energy for the growth of the organism. The acetylation, aryl methyl oxidation and cyclization products of pendimethalin, as reported in *Azotobacter chroococcum* (Holding and Collee, 1971) were not detected in culture filtrate of *Bacillus circulans* (Kole *et al.*, 1994). Megadi *et al.* (2010) reported that pendimethalin degradation with *Bacillus circulans* was by nitroreduction and oxidative N-dealkylation via secretion of pendimethalin nitroreductase and pendimethalin N-dealkylase. The microbial degradation of dinitroaniline herbicides, pendimethalin and trifluralin has been reported to occur most often by oxidative N-dealkylation and nitroreduction. The nitro group reduction and oxidative N-dealkylation destroys the herbicidal activity of pendimethalin, leading to its detoxification (Zayed *et al.*, 1983; Singh and Kulashrestha, 1991; Kole *et al.*, 1994; Megadi *et al.*, 2010).

Toxicity assessment

The remaining toxicity of pendimethalin against *Rhizobium leguminosarum* biovar *viciae* in the aqueous solutions after 28 days after

treatment with tested microbial isolates was estimated in Table 1. The results show that the supernatant of pendimethalin after 28 d treatment with *Bacillus meaterium* (E22) had no antibacterial activity when bioassay was conducted with *Rhizobium leguminosarum* biovar *viciae* as a test organism. The obtained results were compared with control treatment. This implies that the aqueous solution spiked with pendimethalin was completely detoxified after 28 days of treatment with *Bacillus meaterium* (E22) isolate. The bioassay test also reassures the aforementioned obtained results with analysis by using gas chromatograph. Our results are agree with the finding of Bailey and Coffey (1986) who used the same

Table 1: Toxicity of pendimethalin solutions against *Rhizobium leguminosarum* biovar *viciae* after 28 days incubation with *Bacillus meaterium* (E22).

| Treatments | % Inhibition |
|---------------------------------|--------------|
| <i>Bacillus meaterium</i> (E22) | 0 |
| pendimethalin | 100 |

Conclusion

In the present study, bioremediation of pendimethalin-contaminated aqueous medium was studied by addition of pure culture from *Bacillus meaterium* (E22) in 28 days. *Bacillus meaterium* (E22) showed high ability in Pendimethalin degradation. There is no toxicity of pendimethalin detected in aqueous medium after it treated with *Bacillus meaterium* (E22) on *Rhizobium leguminosarum* biovar *viciae*. The results suggest that bioremediation by *Bacillus meaterium* (E22) strain and compost were considered to be the effective method for detoxification of pendimethalin in aqueous system.

REFERENCES

- Appleby, J.R.and Valverde, B.E.(1988).Behavior of dinitroaniline herbicides in plants. Weed Technol. 3: 198–206.
- Baily, A. M.,and Coffey, M. D.(1986).Characterization of microorganisms involved in accelerated biodegradation of metalaxyl and methochlor in soils. Can J Microbio, 32 (7): 562–569.
- Belal, E.B.,Zidan, N.A., Mahmoud, H.A. and Eissa, F.I (2008). Bioremediation of pesticides contaminated soils. J. Agric. Res. Kafrelsheikh Univ. 34: 588 – 608.
- Belal, B. E. and El-Nady M. F. (2013). Bioremediation of pendimethalin-contaminated soil. Afr. J. Microbiol. Res. (in press).
- Bollag, J.M.and Liu, S.Y.(1990).Biological transformation process of pesticides. In Pesticides in the Soil Environment, H.H. Chang, ed., Soil Sci. Soc. Am. (Madison), pp.169–211.
- Chaudhry, G.R., and Ali, H.D.(1988).Bacterial metabolism of carbofuran. Appl. Environ. Microb. 54:1414 - 1419.
- De- Schrijver, A.,and De- Mot, R.(1999).Degradation of pesticides by Actinomycetes. Crit. Rev. Microbiol. 25: 85–119.

- Holding, A.J. and Collee J.G (1971). Routine biochemical tests, in Ribbons (Academic Press, London), 6A, 1 – 32.
- Hong, Q., Zhang, Z., Hong, Y. and Li, S. (2007). A microcosm study on bioremediation of fenitrothion-contaminated soil using *Burkholderia* sp. FDS-1. *Internat. Biodeter. & Biod.* 59: 55–61.
- Jaźwa, A., Szpyrka, E. and Sadlo. (2009). Disappearance of pendimethalin in soil and its residue in ripe fennel. *J. Cent. Europ. Agric.* 10:153 – 158.
- Karpouzias, D.G. and Walker, A. (2000). Factors influencing the ability of *Pseudomonas putida* strain epl and epl1 to degrade the organophosphate ethoprophos. *J. Appl. Microbiol.* 89: 40 – 48.
- Karpouzias, D.G., Fotopoulou, A., Spiroudi, U.M. and Singh, B.K. (2005). Non-specific biodegradation of the organophosphorus pesticides, cadusafos and ethoprophos, by two bacterial strains. *FEMS Microb. Ecol.* 53: 369–378.
- Kole, R.K., Sah, a. J., Pal, S., Chaudhuri, S. and Chowdhury, A. (1994). Bacterial degradation of the herbicide pendimethalin and activity evaluation of its metabolites. *Bull. Environ. Contam. Toxicol.* 52: 779–786.
- Kopytko, M., Chalela, G. and Zauscher, F. (2002). Biodegradation of two commercial herbicides (Gramoxone and Matancha) by the bacteria *Pseudomonas putida*. *Elect. J. Biotechn.* 5: 182 -195.
- Krause, A., Hancock, W.G., Minard, R.D., Freye, R.A.J. and Honeycutt, R.C. (1985). Microbial transformation of the herbicide metolachlor by a soil actinomycete. *J. Agric. Food Chem.* 33: 584–589.
- Kyria, L.B.M., Mervyn, L., De- Souza, T., Raphi, M., Lawrence, P.W. and Michael, L.J.S. (1997). The *atzB* gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Appl. Environ. Microbiol.* 63: 916–923.
- Megadi, V.B., Tallur, P.N., Hoskeri, R.S., Mulla S.I. and Ninnekar, H.Z. (2010). Biodegradation of pendimethalin by *Bacillus Cirulans*. *Indian J. Biotech.* 9: 173 – 177.
- Parka, S.J. and Soper, O.F. (1977). The physiology and mode of action of the dinitroaniline herbicides. *Weed Sci.* 25:79-87.
- Parry, J. M., Turnbull, P. C. B. and Gibson, T. R. (1983). A colour Atlas of *Bacillus* Species. Wolf Medical Books, London.
- Pattanasupong, A., Nagase, H., Inoue, M., Hirata, K., Tani, K., Nasu, M. and Miyamoto, K. (2004). Ability of microbial consortium to remove pesticide, carbendazim and 2,4- dichlorophenoxyacetic acid. *World J. Microbiol. Biotechnol.* 20: 517–522.
- Ross, J.A. and Marco, G.J. (1978). CGA-48988-potatoes residues observed and metabolism data including the analytical methods used. Report no. ABR-78055. Agricultural Division, CIBA-Geigy Corp., Greensboro, N.C.
- Saha J, Chowdhury A. and Chaudhuri S (1991). Stimulation of heterotrophic dinitrogen fixation in barley root association by the herbicide pendimethalin and its metabolic transformation by *Azotobacter* spp. *Soil Biol. Biochem.* 23: 569–574.
- Sambrook, J., Fritsch, E.F. and Maniatis, J. (1989). Molecular cloning. In: A Laboratory Manual (2nd ed.). USA: Cold Spring Harbor Laboratory.

- Singh, S.B. and Kulashrestha, G.(1991).Microbial degradation of pendimethalin. J. Environ. Sci. Health B. 26: 309 – 321.
- Smith, A.E., Aubin,A.J., McIntosh,T.C.(1995). Field persistence studies with emulsifiable concentrate and granular formulations of the herbicide pendimethalin in Saskatchewan. J. Agric. Food Chem. 43: 2988–2991.
- Spain, J.C.andNishino,S.F.(1987).Degradation of 1,4-dichlorobenzene by a *Pseudomonas* sp. Appl. Environ. Microbiol. 53: 1010–1019.
- Strandberg, M., Scott-Fordsm and, J.J. (2004). Effects of pendimethalin at lower trophic Levels-a review. Ecotoxicol. Environ. Saf. 57: 190–201.
- Tiainen, J.A.h., Valo, R., Jarvinen, M. and Joutti, A.(2002).Microbial toxicity tests and chemical analysis as monitoring parameters at composting of creosote-contaminated soil. Ecotoxicol. Environ. Saf. 53: 323–329.
- Vidali, M .(2001).Bioremediation. An overview. Pure Appl. Chem. 73: 1163–1172.
- Zayed, S.M., Mostafa, I.A., Farghaly, M.M., Attaby, H.S., Adam, Y.M.and Mahdy, F.M. (1983).Microbial degradation of trifluralin by *Aspergillus carneus*, *Fusarium oxysporum* and *Trichoderma viride*. J. Environ. Sci. Health. B. 18: 253 – 267.

إزالة مبيد الحشائش بينداميثالين بواسطة البكتريا العصوية *Bacillus megaterium*

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تم عزل و التعرف على احد سلالات الكائنات الحية الدقيقة وهي نوع من البكتريا العصوية *Bacillus megaterium* (E22) من التربة التي سبق معاملتها بمبيد الحشائش بينداميثالين باستخدام تقنية enrichment technique . وعند دراسة تأثير درجة الحموضة ودرجة الحرارة على قدرة نمو السلالة المختبر، أظهرت النتائج أن درجة الحموضة ودرجة الحرارة المثلى لنمو هذه السلالة التي لها القدرة على تحطيم مبيد بينداميثالين هي 7 و 30 درجة مئوية على التوالي. واستخدمت هذه البكتريا في ازالة مبيد بينداميثالين من البيئة السائلة المعدنية و كانت فترة عمر نصف مبيد بينداميثالين 5.6 يوما بينما كانت في الكنترول 53,4 يوما في البيئة السائلة المعدنية الغير معالجة. ولا يوجد أي سمية لمبيد بينداميثالين على بكتريا العقد الجذرية في الفول *Rhizobium leguminosarum* بواسطة اختبار الكائنات الحية الدقيقة في المحلول بعد 28 يوما من معاملتها بالبكتريا *B. megaterium* . وتشير النتائج إلى أن المعالجة البيولوجية بواسطة بكتريا *B. megaterium* التي تم عزلها تعتبر طريقة فعالة لإزالة سمية مبيد بينداميثالين من البيئة المائية.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة

كلية الزراعة – جامعة كفر الشيخ

أ.د / عادل عبد المنعم

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