Soil Environ. 32(2): 96-102, 2013
www.se.org.pk
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Diazinon decomposition by soil bacteria and identification of degradation products by GC-MS

***Tamer Mohamed Ahmed Mohamed Thabit*[*1,2] *and Medhat Ahmed Hassan El-Naggar*[2,3]**
[1]Central Agriculture Pesticides Lab. (CAPL), Agriculture Research Center, Giza, Egypt
[2]Central Lab. of Research, Grain Silos & Flour Mills Organization (GSFMO), Riyadh, KSA
[3]Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt

Abstract

Bacterial degradation trial for diazinon residues was performed using bacterial strains labeled as S1, S2, S3, S4 and S5 isolated from newly reclaimed agricultural soil. Diazinon residues were determined at successive intervals untill 30 days after incubation in liquid medium containing bacteria paralleled with control samples. Diazinon recovery rate was carried out at 0.1 and 1 mg kg^{-1} levels; values were 95.77 and 101.12%, respectively, limit of detection (LOD) was 0.01 mg kg^{-1}, while limit of quantification (LOQ) was 0.03 mg kg^{-1}. Diazinon half-life values (RL_{50}) were 9.36, 8.57, 12.71, 7.51 and 8.20 days for S1, S2, S3, S4 and S5, respectively, while control value was 14.06 days. No significant effect on diazinon occurred with S3 (Staphylococcus sciuri) treatment, while S1 (Pseudomonas aeruginosa), S2 (Bacillus amyloliquefaciens), S4 (Bacillus pseudomycoides) and S5 (Bacillus licheniformis) treatments showed significant effect, that increased diazinon degradation rate compared to control treatment. Only one degradation product was detected from bacterial degradation namely 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP).

Keywords: *Insecticides, biodegradation, bacteria, agricultural soil, degradation products*

Introduction

Pesticides in different chemical moieties that are used for crop protection are considered to be most widely distributed contaminants in the environment over the last century. Million tons of pesticides are produced and spread annually worldwide (Courdouan *et al.,* 2004). Due to widespread use, environmental matrices such as water, soil and air are exposed to pesticides in large quantities (Kim and Smith, 2001; Zulin *et al.,* 2002; Rudel and Perovich, 2009). Terrestrial ecosystems, particularly soil and water, receive large amounts of pesticides during handling and application (Singh *et al.,* 2004). Due to the magnitude of this problem and the lack of a reasonable solution, rapid, effective and ecologically responsible cleaning up method is greatly needed to degrade toxic organopollutants (Boopathy, 2000; Mayer and Staples, 2002). Mechanisms for cleanup of pesticides in soil such as chemical treatment, volatilization and incineration have met public opposition because of the use of large volumes of acids and alkalis (Lau et al., 2003). Generally, physical and chemical cleanup technologies are expensive and sometimes not much effective. Biological techniques such as bioremediation by utilizing microorganisms have been proven very effective (Levin and Forchiassin, 2003; Schoefs *et al.,* 2004). Some microorganisms whether bacteria or fungi, show the capability to degrade pesticides through specific pathways by using them as carbon and energy sources (Aislabie and Lloyd-Jones, 1995).

Among various groups of pesticides, organophosphates are commonly used and some bacterial strains have shown the ability to convert these pesticides into sulfons or oxons or some other degradation products (Hill, 2003). Diazinon [O, O-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate] is commonly used Organophosphorous insecticide. It is cholinesterase inhibitor and acts as non-systemic insecticide and acaricide with contact, stomach, and respiratory action. It is commonly used to control many insect pests such as sucking and chewing insects and mites in a wide range of crops, ornamentals, lawns, and for domestic purpose (Tomlin, 2006). An *in vitro* biodegradation study was conducted to examine the capability of some bacterial strains existing in the newly reclaimed agricultural soil to degrade diazinon to less toxic degradation products. Five common bacterial strains were selected and identified from soil after screening, inoculated in a pure liquid culture media fortified with a known amount of diazinon and incubated. Samples were taken at sequential intervals till 30 days parallel with control sample. Diazinon degradation products were monitored by GC-MS to define the main degradation products resulting from bacterial degradation.

*Email: dr.tamerthabit@live.com

Materials and Methods

Sample collection and preparation

Sandy loam soil samples, which were used for screening and isolation of bacterial strains, were collected from the soil surface layer (0-10 cm), from five different areas at newly reclaimed lands, Nubariya area, Egypt, during the growing season of 2010. Samples were placed into Sterilin polyethylene bags, then air dried for 3-5 day at 18 °C and sieved through 2 mm sieve to be representative and homogeneous. The samples were kept at 4 °C until use.

Bacterial isolation and identification

Isolation of microorganisms was carried out with Soil dilution technique. Ten gram representative soil samples (three replicates) were shaken in 90 mL of 0.01% agar in sterile water for 10 min., then left standing for further 20 min. Dilution series was made up to 10^6, then aliquots (0.5 mL) were spread on Czapek-dox agar medium (CZA, 45.4 g L^{-1} purified water), Biolog Inc., California, USA. Plates were amended with Triton X-100 (2 mL L^{-1}) as a spreading agent and incubated at 33±2 °C for 2 weeks (Jones and Stewart, 1997). Bacterial isolation was carried out using nutrient agar as a selective medium (Charlau Chemicals, Spain). After dilution of soil samples (10^{-2}-10^{-12}), selective agar media was inoculated and incubated at 33±2 °C, for bacterial strain selection; Colonies grow within 48-72 hours in usual (Ilyina *et al*., 2003). Bacterial strains were characterized by determining their utilization profiles on microtiter plate designed to test the ability of an organism to oxidize 95 different carbon sources using BIOLOG GIN III system, Biolog Inc., California, USA. Bacterial isolates were grown for 24 hours at 33±2 °C on Biolog Universal Growth Agar medium (BUG, 57 g L^{-1} purified water) supplemented with 5% sheep blood, according to Civilini (2009). Sub trial was performed to define the proper diazinon concentration for optimum bacterial growth, by determining bacterial growth in BUG medium supplied with diazinon as sole carbon source in different concentrations (0, 10, 20, 50, 100, 200 mg kg^{-1}). Rate of bacterial growth was estimated based on standard plate count technique by direct determination of viable cell count per mL using plate count agar medium (PCA, 23.5 g L^{-1} purified water), from Charlau Chemicals, Spain.

Working sample preparation

Five common bacterial strains were selected and identified from more than 20 strains. Known concentration of diazinon active ingredient (a.i.) 97.50%, from Dr. Ehrenstorfer Reference Materials, Germany, was used to prepare working solution (5 µg diazinon a.i. for one mL liquid medium) and was spread in a sterile culture tube, solvent was evaporated under pure nitrogen stream, then BUG pure liquid medium (57 g L^{-1} purified water), pH 7.3±0.1 was added (9 mL). Bacterial strains were inoculated each separately (1 mL), tubes were shaken for 30 min, then incubated at 33±2 °C. Samples were taken at successive intervals after incubation at zero, 3 hours, 1, 2, 4, 7, 10, 15, 21 and 30 days paralleled with control samples at each interval. Zero time is the initial concentration directly before incubation (50 µg mL^{-1}).

Residues extraction and cleaning up procedures

Solid Phase Extraction (SPE) technique was used for extraction and cleaning-up of diazinon residues from the liquid medium after volume modifications of the procedure mentioned by López-Blanco *et al*. (2006). CUPSA3SPE cartridge (C18+n-2aminoethyl, 100 mg mL^{-1}) from United Chemical Technologies (UCT), USA, was conditioned with ethyl acetate (5 mL) followed by methanol (5 mL) and ultrapure water (5 mL) at rate of 3 mL min^{-1}, without allowing the cartridge to dry out. The aqueous sample (10 mL) was loaded on and passed through the cartridge at rate of 0.8 mL min^{-1} (sample was filtered before loading to remove suspended and insoluble materials of bacteria). Cartridge was dried by nitrogen stream (purity 99.999%) over surface for 2 min. Adsorbed pesticide was eluted by ethyl acetate (5 mL). Rapid Trace SPE workstation from Zymark, Caliper Life Sciences was used for SPE handling; solvents used were HPLC grade, from BDH chemicals, UK. Agilent 7890A gas chromatography equipped with nitrogen–phosphorus detector (NPD) and HP-5 capillary column (30 m × 320 µm × 0.25 µm) from J&W Scientific was used for pesticide residue analysis, injector was at 260 °C, splitless mode, detector at 320 °C, ignition gases H_2 at 3 mL min^{-1} and air at 45 mL min^{-1}. Oven programmed at 120 °C for one min, ramped at 20 °C min^{-1} to 270 °C and held for 2 min., carrier gas N_2 was kept at flow rate 3 mL min^{-1}. Diazinon retention time (Rt) was 3.71 min. Perkin Elmer (PE) GC-MS, Clarus 500, electron impact ionization (EI) equipped with Elite-5MS capillary column (30 m × 250 µm × 0.25 µm) was used for detection of diazinon degradation products, Injector at 260 ºC, splitless mode, Helium was used as carrier gas at flow rate of 1.0 mL min^{-1}, oven was programmed at 80 °C for 2 min., ramped at 10 °C min^{-1} to 280 °C, held for 8 min., MS source and transfer line temperatures were 230 and 280 ºC, respectively.

Method validation studies

Stock solution of diazinon standard (400 ng μL^{-1}) freshly prepared in ethyl acetate was used for calibration and calculation. Working solutions at 1, 2, 5, 10, 20, 40 mg L^{-1} were prepared. Recovery rate was performed using

untreated liquid media, which was used in treatments and spiked with diazinon a.i., solution at two levels 0.1 and one mg kg^{-1}, and procedures of mentioned entire method were performed. Recovery values achieved were 95.77 and 101.12%, respectively. Estimated values of the limit of determination (LOD) and limit of quantitation (LOQ) of the analytical method used were 0.01 and 0.03 mg kg^{-1}, respectively.

Kinetic studies

The degradation rate of diazinon was calculated mathematically according to Timme and Frehse (1980), that degradation behavior of pesticide residues can be described mathematically as a pseudo-first order reaction; rate of degradation (K) could be calculated using common logarithms from the following equation:

$$\log R = \log R_0 - 0.434Kt$$

***R_0*:** residue level at the initial time (zero time),
***R*:** residue level at interval in days after application
***Kt*:** degradation rate constant at the successive intervals in days
K: mean of *Kt*

Diazinon half-life value (RL_{50}) was calculated mathematically according to Moye et al. (1987) from the following equation:

$$RL_{50} = \frac{Ln2}{K}$$

Statistical analysis

t-Test was used for analyzing the obtained data statistically to define the significance levels with the basis outlined by Snedecor and Cochran (1967).

Results and Discussion

Bacterial strains identification

Morphology and characteristics of the selected bacterial strains are presented in Table 1. The strain S1 (*Pseudomonas aeruginosa*) was a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It had the ability to grow at 42 °C. Strain S2 (*Bacillus amyloliquefaciens*) was Gram-positive with catalase positive activity and aerobic in nature. This bacterium was rod-shaped and motile. It also had the ability to degrade proteins extracellularly by excreting a particular enzyme called subtilisin. It is useful in some industrial processes such as laundry detergents and contact lens cleansers. *Staphylococcus sciuri* (S3) was Gram positive, oxidase-positive, coagulase-negative, originally used to categorize 35 strains shown to utilize cellobiose, galactose, sucrose and glycerol. Strain S4 (*Bacillus pseudomycoides*) is Gram positive, non-motile, forms chains of cells and can hydrolyze starch, casein and gelatin. Likewise, S5 (*Bacillus licheniformis*) was also Gram positive. It is commonly found in soil and on bird feathers. Moreover, it is a thermophilic bacterium, optimal growth temperature is around 30°C and can survive at much higher temperatures as well.

Diazinon residues

Table 2 & Figure 1 show the data of diazinon residues as µg mL^{-1} found after incubation with tested bacterial strains. The strains S1, S2, S4 and S5 were found to be effective for the diazinon degradation, while S3 had non-significant effect on bacterial diazinon degradation. Calculated half-life values (RL_{50}) of diazinon inoculated with S1, S2, S4 and S5 were 9.36, 8.57, 7.51 and 8.20 days, respectively, while in case of S3 half-life was12.71. Half life of the control was 14.06 days. *t* -Values at significance level $p \leq 0.05$ were 0.0022, 0.0020, 0.0801, 0.0010 and 0.0017 for S1, S2, S3, S4 and S5, respectively.

The strain S4 was the most effective strain for diazinon degradation, followed by S5, S2 and S1. Results of this study clearly demonstrated that the *Bacillus* and *Pseudomonas* sp. had a significant influence on diazinon degradation rate, however Cycon *et al.* (2009) found *Serratia* and *Pseudomonas* spp. Efficient for the bioremediation of diazinon-contaminated soils. Thabit and El-Naggar (2013) reported similar results that some bacterial strains isolated from the agricultural soil, namely *Pseudomonas aeruginosa*, *Bacillus pseudomycoides* and Bacillus licheniformis have the ability to degrade malathion insecticide. Ghosh Poorva *et al.* (2010) reported the

Table 1: Morphology and characteristics of tested bacterial strain

Strain	Colony Name	Shape	Color	Gram Character	Motility
S1	*Pseudomonas aeruginosa*	flat and irregular	yellow-green and fluorescent	negative	Unipolar motility
S2	*Bacillus amyloliquefaciens*	spreading and irregularly	Creamy	positive	Motile
S3	*Staphylococcus sciuri*	Circular	Yellowish -gray	positive	Non-motile
S4	*Bacillus pseudomycoides*	Singly and short chains	white to cream	positive	Non-motile
S5	*Bacillus licheniformis*	round, matt and granular	greenish	positive	Motile

Table 2: Diazinon (µg mL^{-1}) found after incubation with tested bacterial strains

Time	C		S1		S2		S3		S4		S5	
	Residue	%Loss	Residue	%Loss	Residue	%Loss	Residue	%Loss	Residue	%Loss	Residue	%Loss
0	50.00	00.00	50.00	00.00	50.00	00.00	50.00	00.00	50.00	00.00	50.00	00.00
3h	49.71±0.18	0.57	49.59±0.22	0.82	49.68 0.11	0.64	49.63±0.21	0.73	49.74±0.20	0.52	49.73±0.19	0.54
1	47.66±0.15	4.67	46.23±0.38	7.53	46.67±0.50	6.65	46.88±0.33	6.23	44.86±1.66	10.27	46.82±0.57	6.35
2	44.73±0.81	10.53	42.38±1.81	15.24	41.16±0.94	17.67	43.64±0.49	12.71	37.74±1.43	24.52	39.34±1.05	21.32
4	40.72±0.24	18.55	36.68±0.89	26.63	34.96±0.64	30.08	40.14±0.38	19.72	32.46±1.02	35.08	31.64±0.84	36.72
7	35.06±36	29.88	30.87±0.57	38.26	26.65±0.53	46.70	35.92±0.76	28.16	25.64±1.32	48.72	24.70±0.48	50.60
10	30.60±0.28	38.80	24.73±1.12	50.53	20.86±0.98	58.28	30.59±1.87	38.82	19.04±0.49	61.91	19.94±0.34	60.12
15	24.83±0.50	50.33	18.60±1.14	62.80	14.89±1.33	70.21	24.35±1.52	51.29	13.59±0.79	72.81	15.30±0.98	69.40
21	18.72±0.47	62.56	9.74±0.68	80.51	9.62±0.74	80.76	17.84±0.37	64.32	9.18±0.46	81.64	10.87±0.85	78.25
30	11.33±0.86	77.33	4.81±0.45	90.37	4.11±0.39	91.78	10.85±0.69	78.29	5.81±0.47	88.37	5.67±0.32	88.66
K	0.04927		0.07397		0.08079		0.05449		0.09227		0.08450	
RL_{50} (days)	14.06		9.36		8.57		12.71		7.51		8.20	
T p≤0.05	----		0.0022		0.0020		0.0801		0.0010		0.0017	

*: Zero time (the initial concentration before incubation); **: Samples were taken three hour after incubation;
RL_{50}: Half-life value; K: Degradation rate constant; *p*: significance level at $p \leq 0.05$, Mean± Standard Deviation (SD)

degradation of methyl parathion (phosphorothiolothionate), by using *Pseudomonas* species. Forrest *et al.* (1981) reported that *Flavobacterium* sp. isolated from soil had the diazinon degrading ability in neutral phosphate buffer. Kanekar Pradnya *et al.* (2004) indicated that *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* have the ability to utilize organophosphorous pesticides as carbon and energy source. Horne *et al.* (2002) found that *Agrabacterium radiobacter* was able to hydrolyze a wide range of organophosphorus insecticides. Similarly, Deshpande *et al.* (2001) reported that *Pseudomonas aeruginosa* MCMB-427 had ability to degrade dimethoate insecticide.

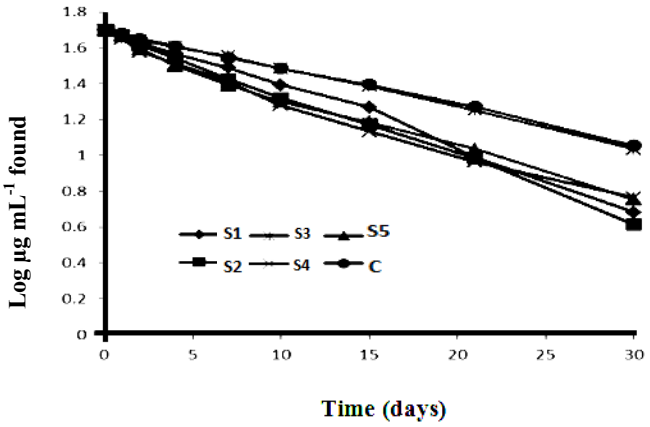

Figure 1: Diazinon biodegradation rate by tested bacteria strains

Degradation products identification

Diazinon insecticide is a phosphorothionate moiety (Figure 2), which belongs to the main chemical group of organophosphorous (Hassal, 1990). 2-Iso-4-methyl-6-hydroxypyrimidine (IMHP) was identified as the main degradation product of diazinon through GC/MS analysis after 10, 21 and 30 days of incubation (Figure 3). Retention time for the diazinon was 13.86 min and it had molecular formula $C_{12}H_{21}N_2O_3PS$ with molecular weight of 304.3 kDa. Similarly IMHP, the main degradation product was detected at R_t 10.06 min. with molecular formula of $C_8H_{12}N_2O$ and molecular weight of 152.19 kDa. Previously, scientists have reported 2-isoprophyl-4-methyl-6-hydroxypyrimidine as the degradation product of diazinon (Forrest *et al*., 1981; Sovcool *et al.,* 1981; Kouloumbos *et al.,* 2003).

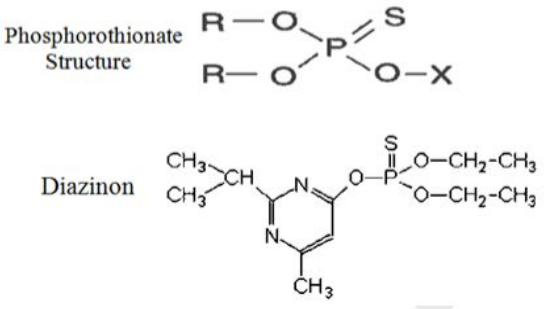

Figure 2: Phosphorothionate and diazinon chemical structure

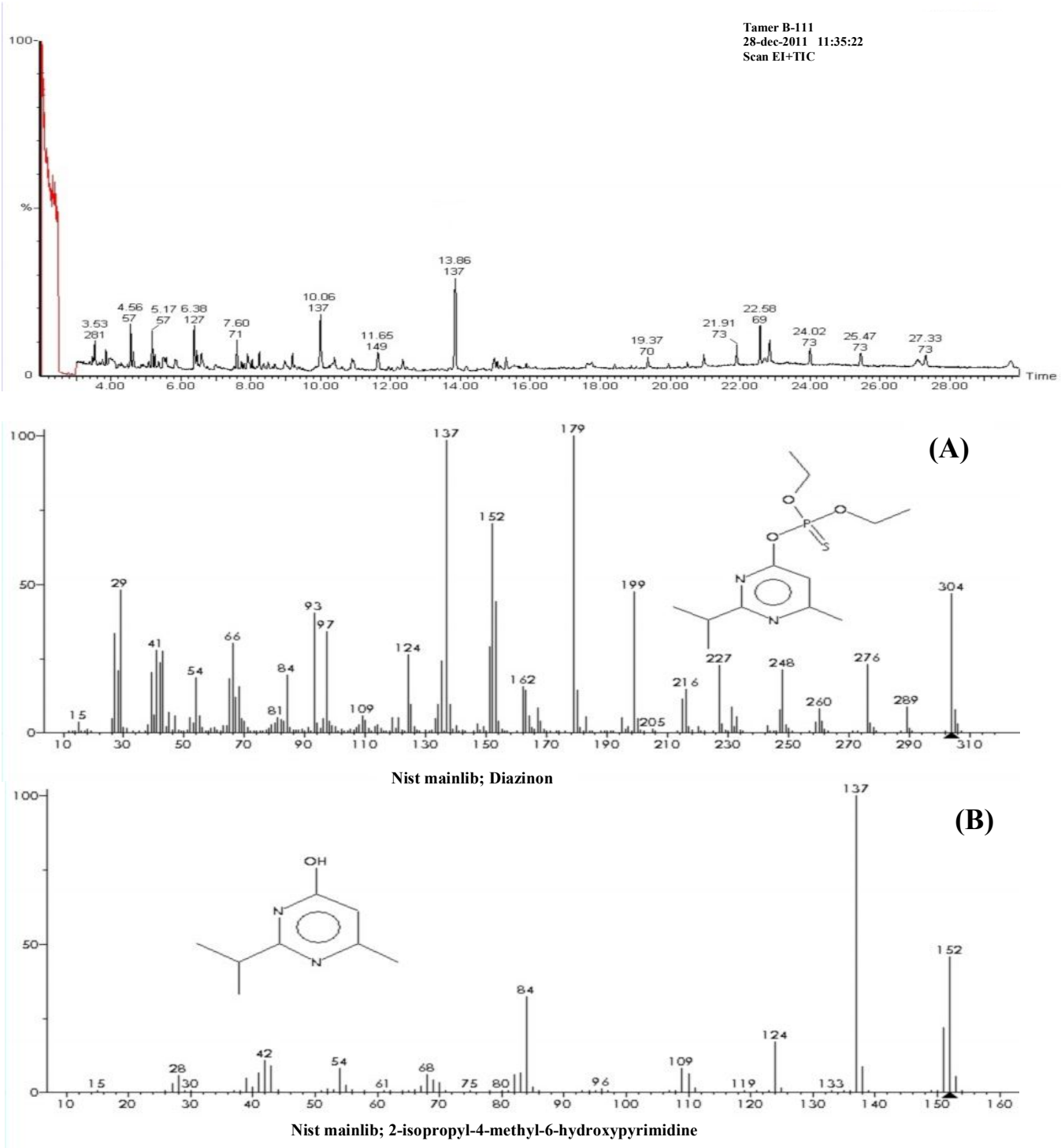

Figure 3: Mass spectrum of Diazinon (A) and IMHP (B) by GC/MS

Diazinon released into the environment is moderately persistent and mobile (Howard, 1991; EPA, 2006). Diazinon application to soils showed that it is not likely to adsorb on soils. Similarly, diazinon was found to be mobile in 80% of the tested soil samples. This can enhance the leaching of diazinon, especially in light-textured soils with low organic matter content (EPA, 2000). Microbial degradation in soils is the primary route of diazinon dissipation from the environment. Diazoxon is the primary product of degradation in case of diazinon hydrolysis. However, diazoxon is rapidly hydrolyzed to oxypyrimidine which is more mobile in the environment than the parent compound (EPA, 2000). The present study clearly showed that the selected strains could be used for the degradation of diazinon pesticide in the environment.

Conclusion

The bacterial strains isolated from the agricultural soil, especially *Pseudomonas* and *Bacillus* species showed the ability to degrade diazinon insecticide. Identified degradation product of the bacterial biodegradation was 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP). Overall, this study provides the complete insight of the diazinon degradation with the help of bacterial strains, which could be helpful to devise a proper strategy for the degradation of pesticides under field conditions.

Acknowledgement

We are grateful to Dr. Dalia I.H. El-Geddawy (Sugar Crop Res. Inst., Agriculture Research Center, Egypt) for review and revision of this manuscript.

References

Aislabie, J. and G. Lloyd-Jones. 1995. A review of bacterial degradation of pesticides. *Australian Journal of Soil Research* 33: 925-942.

APVMA. 2003. The Reconsideration of Registrations of Products containing Diazinon and their Labels; Review Report, Review Series 1, Part 1 Product Cancellations. Australian Pesticides & Veterinary Medicines Authority. Canberra, Australia.

Bavcon, M., P. Trebse and L. Zupancic-Kralj. 2003. Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionisation detector. *Chemosphere* 50: 595-601

Boopathy, R. 2000. Bioremediation of explosives contaminated soil. *International Biodeterioration and Biodegradation* 46: 29-36.

Civilini, M. 2009. Identification and characterization of bacteria isolated under selective pressure of volatile organic compounds. *Journal of Environmental Biology* 30(1): 99-105.

Courdouan, A., S. Marcacci, S. Gupta and J.P. Schwitzguebel. 2004. Lindane and technical HCH residues in Indian soils and sediments: a critical appraisal. *Journal of Soils and Sediments* 4(3): 192-196.

Cycon, M., M. Wojcik and Z. Piotrowska-Seget. 2009. Biodegradation of the organophosphorus insecticide diazinon by *Serratia* sp. and *Pseudomonas* sp. and their use in bioremediation of contaminated soil. *Chemosphere* 76(4): 494-501.

Deshpande, N.M., P.K. Dhakephalkar and P.P. Kanekar. 2001. Plasmid-mediated dimethoate degradation in *Pseudomonas aeruginosa* MCMB-427. *Letters in Applied Microbiology* 33(4): 275–279.

EPA. 2000. Environmental Risk Assessment for Diazinon; U.S. Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office. Washington DC.

EPA. 2006. Reregistration Eligibility Decision (RED) Diazinon; Environmental Protection Agency (EPA) 738-R-04-006. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office. Washington DC.

Forrest, M., K.A. Lord, N. Walker and H.C. Woodville. 1981. The influence of soil treatments on the bacterial degradation of diazinon and other organophosphorus insecticides. *Environmental Pollution Series A, Ecological and Biological* 24(2): 93-104.

Ghosh Poorva, G., A. Sawant Neha, S.N. Patil and B.A. Aglave. 2010. Microbial biodegradation of organophosphate pesticides. *International Journal of Biotechnology and Biochemistry* 6(6): 871-876.

Hassal, A.K. 1990. Organophosphorous insecticides, The Biochemistry and Uses of Pesticides. ELBS publication, London. P. 81-124.

Hill, E.F. 2003. Wildlife toxicology of organophosphorus and carbamate pesticides. p. 281-312. *In:* Handbook of Ecotoxicology. D.J. Hoffman, B.A. Rattner, G.A. Burton Jr. and J. Cairns Jr. (eds.). Lewis Publishers, Boca Raton.

Horne, I., T.D. Sutherland, R.L. Harcourt, R.J. Russell and J.G. Oakeshott. 2002. Identification of an opd (organophosphate degradation) gene in an *Agrobacterium* isolate. *Applied and Environmental Microbiology* 68(7): 3371-3376.

Howard, P.E. 1991. Handbook of Environmental Fate and Exposure Data for Organic Chemicals; Lewis Publishers, Inc. Chelsea. MI Vol. III. p. 209-221.

Ilyina, A., S.M.I. Castillo, S.J.A. Villarreal, E.G. Ramirez and R.J. Candelas. 2003. Isolation of soil bacteria for bioremediation of hydrocarbon contamination. *ВЕСТН. МОСК. УН-ТА. СЕР. 2. ХИМИЯ* 44(1): 88-91.

Jones, E.E. and A. Stewart. 1997. Biological control of *Sclerotinia minor* in lettuce using *Trichoderma* species. p. 154-158. *In:* Proceedings of the 50th New Zealand Plant Protection Conference. August 18-21, 1997, Lincoln, New Zealand

Kanekar Pradnya, P., J. Bhadbhade Bharati, M. Deshpande Neelima and S. Sarnaik Seema. 2004. Biodegradation of organophosphorous pesticides. *Proceedings of the Indian National Science Academy* B70(1): 57-70.

Kim, J.H. and A. Smith. 2001. Distribution of organochlorine pesticides in soils from South Korea. *Chemosphere* 43(2): 137-140.

Kouloumbos, V.N., D.F. Tsipi, A.E. Hiskia, D. Nikolic and R.B. van Breemen. 2003. Identification of photocatalytic degradation products of diazinon in TiO_2 aqueous suspensions using GC/MS/MS and LC/MS with quadrupole time-of-flight mass spectrometry. *Journal of the American Society for Mass Spectrometry* 14(8): 803-817.

Lau, K.L., Y.Y. Tsang and S.W. Chiu. 2003. Use of spent mushroom compost to bioremediate PAH contaminated samples. *Chemosphere* 52: 1539-46

Levin, L.A. and V.A. Forchiassin. 2003. Degradation of organic pollutants by the white rot *Basidiomycete Trametes trogii*. *International Biodeterioration and Biodegradation* 52: 1-5.

Lopez-Blanco, C., S. Gomez-Alvarez, M. Rey-Garrote, B. Cancho-Grande and J. Simal-Gandara. 2006. Determination of pesticides by solid phase extraction followed by gas chromatography with nitrogen-phosphorous detection in natural water and comparison with solvent drop microextraction. *Analytical and Bioanalytical Chemistry* 384: 1002-1006.

Mayer, A.M. and R.C. Staples. 2002. Laccase: new functions for an old enzyme. *Phytochemistry* 60: 551-65.

Moye, H.A., M.H. Malagodi, J. Yoh, G.L. Leibee, C.C. Ku and P.G. Wislocki. 1987. Residues of avermectin B1a rotational crop and soils following soil treatment with (14C) avermectin B1a. *Journal of Agriculture and Food Chemistry* 35: 859-864.

Rudel, R.A. and L.J. Perovich. 2009. Endocrine disrupting chemicals in indoor and outdoor air. *Atmospheric Environment* 43(1): 170-181.

Schoefs, O., M. Perrier and R. Samson. 2004. Estimation of contaminant depletion in unsaturated soils using a reduced-order biodegradation model and carbon dioxide measurement. *Applied Microbiology and Biotechnology* 64: 256-61.

Singh, K., K. Brajesh, A. Walker, J. Alum, W. Morgan and D.J. Wright. 2004. Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in biodegradation of contaminated soils. *Applied and Environmental Microbiology* 70: 4855-4863.

Snedecor, G.V. and W.G. Cochran. 1967. Statistical Methods. 6th Ed. Iowa State University Press Ames. Iowa, USA.

Sovocool, G.W., R.L. Harless, D.E. Bradway, L.H. Wright, E.M. Lores and L.E. Feige. 1981. The Recognition of Diazinon, an organophosphorus pesticide, when found in samples in the form of decomposition products. *Journal of Analytical Toxicology* 5(2): 73-80.

Thabit, T.M.A. and M.A.H. El-Naggar. 2013. Malathion degradation by soil isolated bacteria and detection of degradation products by GC-MS. *International Journal of Environmental Sciences* 3(5): 1467-1476.

Timme, G. and H. Frehse. 1980. Statistical interpretation and graphic representation of the degradation behaviour of pesticide residues. *Pflanzenschutz Nachrichten Bayer* 33(1): 47-60.

Tomlin, C.D.S. 2006. The Pesticide Manual, A World Compendium. 14th Ed. British Crop Protection Council (BCPC), Alton, Hampshire, UK.

Zulin, Z., H. Huasheng, W. Xinhong, L. Jianqing, C. Weiqi and X. Li. 2002. Determination and load of organophosphorus and organochlorine pesticides at water from Jiulong River Estuary, China. *Marine Pollution Bulletin* 45(1-12): 397-402