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Isolation of Chlorpyriphos and Carbofuran Degrading Bacteria from Pre-treated Soils

M. Nafeesa*, K. Ramaraju, S. Kuttalam and P. Doraisamy
Tamil Nadu Agricultural University, Coimbatore
Malikapparambu, Alathur (PO), Palakkad– 678541, Kerala

Investigations were carried out at Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore to isolate chlorpyriphos and carbofuran degrading bacterial isolates from pre-treated soils. Five each of chlorpyriphos and carbofuran degrading bacterial isolates were obtained from the enrichment cultures. Chlorpyriphos degrading bacterial isolates *viz.,* CPY-1, CPY-2, CPY-3 and CPY-4 were tentatively identified as *Serretia* sp., *Pseudomonas* sp., *Klebsilla* sp. and *Acinetobacter* sp., respectively. Carbofuran degrading bacterial isolates *viz.,* CF-1, CF-2 and CF-7 were tentatively identified as *Enterobacter* sp., *Bacillus* sp. and *Bacillus* sp., respectively. The identity of CPY-5, CF-3 and CF-4 was not confirmed. Based on nucleotide homology and phylogenetic analysis the potential isolates CF-7 and CPY-4 were confirmed as *Bacillus subtilis* and *Acinetobacter sp.* respectively.

Key words: Chlorpyriphos, Carbofuran, Bacterial isolates, Enrichment culturing, Isolation

Organophosphate and carbamate insecticides were introduced to replace the recalcitrant and hazardous chlorinated insecticides. Although these newly introduced insecticides were considered to be biodegradable, some of them are highly toxic and their residues are found in certain environments. Microbial metabolism of organophosphate and carbamate insecticides seems to be the most important in accounting for their degradation in soil (Laveglia and Dahm, 1977). Chlorpyriphos and Carbofuran, the two soil applied insecticides belong to the organophosphate and carbamate group, respectively. They are well known for their residual effect in the soil and water and receive lot of questions concerned with the environment and human health. Since, the earlier studies (Ramanand *et al*., 1988; Chaudry and Ali, 1988; Mallick *et al*., 1999; Sing and Walker, 2002; Sing *et al*., 2004; Ghanem *et al*., 2007; Rani *et al*., 2008) showed that potential microorganisms having the ability to degrade chlorpyriphos and carbofuran are present in the natural environment and these organisms can be effectively utilized for the purpose of detoxifying the soil contaminated with these chemicals. Hence, this study was undertaken to isolate and characterize chlorpyriphos and carbofuran Degrading bacterial isolates from pre-treated soils.

Materials and Methods

Soil samples were collected from the pretreated fields i.e. fields that have the history of application of chlorpyriphos and carbofuran. Sampling was done from the four corners and from the middle of the selected plots at a depth of 15 cm and mixed evenly. After mixing and quartering, 2.5 kg of soil per plot were transferred to clean plastic containers for transport and storage. Samples were not allowed to dry out and handling was kept to minimum to maintain the microbial activity and stored in refrigerator at 4°C for further studies. Different properties of the soil samples *viz*., pH, Electrical Conductivity (EC), organic matter, moisture content and textural class were characterized by following the standard procedures (Mani *et al.,* 2007). The recorded properties are given in Tables 1 and 2.

*Corresponding author email: nafiento@gmail.com

Insecticides used

Two insecticides, one from carbamate group (carbofuran - Furadan®3 G obtained from *FMC India* Private *Limited, Bangalore)* and another from organophosphate group (chlorpyriphos - Dursban®20 EC obtained from Dow Agrochemicals India Private Limited) were selected for conducting the study. Commercial grade of insecticides were used for the enrichment culturing, because it may more closely resemble the active compound that microorganisms are likely to be exposed in the soil environment.

Enrichment culturing

It was done by repeated application of chlorpyriphos and carbofuran at desired intervals to the soil and to the broth cultures. The choice to use high concentration of chlorpyriphos and carbofuran was to enhance the selection pressure, thereby reducing the number of surviving species and only to obtain the organisms that were able to withstand high concentration of these chemicals.

Soil enrichment culturing was done in mud pots having a capacity of 500 g. Before taking the soil samples, the mud pots were washed, sun dried and fumigated with formaldehyde to avoid contamination. Three hundred grams of the soil samples collected from different locations were taken in mud pots. Three replicated pots were maintained for each soil

sample. Soil enrichment was done by following the methodology adopted by Singh *et al*. (2004) and Ramanand *et al.* (1988). The chlorpyriphos pretreated soil samples were further treated with chlorpyriphos (Dursban®) at the rate of 25 µg g^{-1} for ten times at an interval of seven days to maximize the survival of the potential species alone. Similarly, another set of carbofuran pretreated soil samples were further treated with 50 µg of carbofuran (Furadan®3 G) per gram of soil for ten times at seven days interval to maximize the survival of the carbofuran degrading microorganisms. Treated pots were kept at room temperature (30±2°C) during the period of enrichment.

Broth enrichment culturing with chlorpyriphos was done by following the methodology adopted by Rani *et al*. (2008). Air dried and sieved (<2 mm) soil samples (10 g) collected from the chlorpyriphos pre treated fields were suspended in 250 ml conical flasks containing 50 ml of MSM supplemented with chlorpyriphos (50 mg l^{-1}). The flasks were incubated on a shaker (Heco Environment Chamber Cum Shaker, Eurotech Electronic System Model-365) continuously at 250 rpm for seven days at 30°C. Broth enrichment culturing with carbofuran to isolate mixed cultures was done by following the method adopted by Chaudhry and Ali, (1988). Five gram soil samples were suspended in 20 ml of MSM containing 2mg of carbofuran in 100 ml capacity conical flasks. The flasks were incubated for three weeks at 30°C. They were then sub cultured into fresh MSM containing carbofuran. Subculturing was performed periodically at seven days interval, and the concentrations of the carbofuran were gradually increased from 100 to 500 µg ml^{-1} in MSM during subsequent transfers.

Isolation, Purification and characterization

Chlorpyriphos and carbofuran degrading bacterial isolates were isolated both from soil and broth enrichment cultures. One gram of the soil was aseptically removed from the triplicate pots after complete soil enrichment and pooled. From this, one gram of soil was taken as a representative sample for isolation purpose. Serial dilution and plating technique was used to isolate chlorpyriphos and carbofuran degrading bacterial isolates. Serial dilution (10^{-7}) was made with sterile distilled water and one ml of the sample was plated on MS agar medium supplemented with chlorpyriphos or carbofuran (50 µg ml^{-1}) as carbon source. The plates were incubated at 30°C for three days. From the broth enrichment cultures, a loop full of bacterial growth was streaked onto mineral agar supplemented with either chlorpyriphos (for chlorpyriphos degrading isolates) or carbofuran (for carbofuran degrading isolates) at the concentration of 50µg ml^{-1}. The plates were incubated at 30°C for three days. The individual bacterial colonies that grew on the medium were sub cultured onto mineral agar containing either chlorpyriphos or carbofuran (50µg ml^{-1}) until pure cultures of chlorpyriphos and carbofuran degrading bacterial isolates were obtained. Morphological and biochemical characterization of the bacterial isolates were done as per the methods suggested by Cappuccino and Sherman (2002). The isolates were tentatively identified in consultation with "Bergey's Manual of Systematic Bacteriology" and by referring "Microbiology A Laboratory Manual" (Cappuccino and Sherman, 2002). Degradation potentials of the isolates were tested (Nafeesa. M, 2009) and the potential isolates CF-7 and CPY-4 were send to Banglore Genei for identification based on 16S rDNA data.

Results and Discussion

In the present study, bacterial isolates having the ability to degrade chlorpyriphos and carbofuran were isolated from both soil and broth enrichment cultures. Six bacterial isolates *viz.,* CPY-1, CPY-2, CPY-3, CPY-4, CPY-5 and CPY-6 of degrading chlorpyriphos were initially isolated from five soil and broth enrichment cultures. One isolate (CPY-6) lost its ability to grow in the MSA plates during sub culturing. Eight bacterial isolates *viz.,* CF-1, CF-2, CF-3, CF-4, CF-5, CF-6, CF-7 and CF-8 capable of degrading carbofuran were initially isolated from five soil and broth enrichment cultures. Three isolates *viz.,* CF-5, CF-6 and CF-8 lost their ability to grow in the MSA plates provided with carbofuran as the sole source of carbon. In total five chlorpyriphos degrading (CPY-1, CPY-2, CPY-3, CPY-4 and CPY-5) and five carbofuran degrading (CF-1, CF-2, CF-3, CF-4 and CF-7) bacterial isolates were obtained from the pretreated soils (Soils which have >5 years of history of application of chlorpyriphos and carbofuran) after artificial enrichment. The results are in agreement with the hypothesis that the application of pesticides promotes the evolution of microorganisms that are capable of degrading these xenobiotic compounds (Diaz, 2004).

Table 1. Properties of the soil samples collected for chlorpyriphos enrichment cultures

Location	pH	EC (dS/m)	Organic matter (%)	Moisture content (%)	Texture
Nursery soil – Botanical garden, Coimbatore.	7.70	0.41	1.07	27.12	Sandy loam
Cotton field **–** Department of Cotton, Coimbatore	8.20	0.86	0.83	32.02	Clay loam
Rice field – Paddy Breeding Station, Coimbatore.	8.28	0.47	0.95	38.85	Clay loam
Cotton field – Kozhinnampara, Chittoor	7.90	1.02	0.79	29.25	Silt loam
Sunflower field – Department of Oilseeds, Coimbatore.	7.97	1.26	0.88	33.78	Clay loam

Details of chlorpyriphos and carbofuran degrading bacterial isolates related to the growth characteristics in the MSM, type of enrichment culture and history of the soil sample regarding the application of chemicals are listed in Table 3 & Table 4. Morphologiacal and biochemical charecteristics of the above isolates are listed in Table 5 & 6. Chlorpyriphos degrading bacterial is3 olates (CPY-1 and CPY-4) and carbofuran degrading bacterial isolates (CF-1, CF-2, CF-4 and CF-7) developed clear zones on solid synthetic medium provided with chlorpyriphos and carbofuran, respectively as the sole source of carbon when compared to the other isolated strains. These clear zones can be explained by the liberation of extra cellular enzymes produced by the microbial cells (Slaoui *et al*., 2007). Clark and Wright (1970) isolated *Arthrobacter* and *Achromobacter* sp., utilizing a herbicide (Isopropyl-N-phenyl carbamate) from the soil and suggested that the process of clear zone formation was due

Table 2. Properties of soil samples collected for carbofuran enrichment cultures

Location	pH	EC (dS/m)	Organic matter (%)	Moisture content (%)	Texture
Banana field – Onampalayam, Coimbatore	7.70	0.38	1.02	25.60	Clay loam
Banana field – Kannara, Trichur	5.28	0.14	1.24	28.23	Silt loam
Rice field – Paddy Breeding Station, Coimbatore	8.20	0.42	0.90	39.48	Clay loam
Banana field – Orchard, Coimbatore.	7.78	1.64	0.93	19.58	Clay loam
Corn field – Department of Millets, Coimbatore.	7.81	1.80	0.84	22.90	Clay loam

Table 3. Details of chlorpyriphos degrading bacterial isolates from Tamil Nadu and Kerala

Isolate	Growth characteristics on MSA provided with chlorpyriphos as the sole source of carbon	Type of enrichment culturing from which the isolates were obtained	History of the soil regarding the application of the chlorpyriphos	Location
CPY-1	Slimy dull coloured growth with hallowing around the colonies	Broth enrichment	> 2 years	Nursery soil, Coimbatore
CPY-2	Flat wrinkled colonies with yellow tinge	Soil enrichment	>10 years	Rice field, Coimbatore
CPY-3	Dull coloured raised translucent colonies	Broth enrichment	> 6 years	Cotton field, Chittoor
CPY-4	Shiny bluish colonies with hallowing around the growth	Broth enrichment	> 10 years	Cotton field, Coimbatore
CPY-5	Dull coloured, slimy raised colonies	Broth enrichment	> 10 years	Sunflower field, Coimbatore
CPY-6	Grayish black coloured pin pointed colonies	Soil enrichment	> 10 years	Rice field, Coimbatore

CPY-1 (Chlorpyriphos degrading isolate-1); CPY-2 (Chlorpyriphos degrading isolate-2); CPY-3 (Chlorpyriphos degrading isolate-3) CPY-4 (Chlorpyriphos degrading isolate-4); CPY-5 (Chlorpyriphos degrading isolate-5); CPY-6 (Chlorpyriphos degrading isolate-6)

Table 4. Details of carbofuran degrading bacterial isolates from Tamil Nadu and Kerala

Isolate	Growth characteristics on MSA provided with carbofuran as the sole source of carbon	Type of enrichment culturing from which the isolates were obtained	History of the soil regarding the application of the carbofuran	Location
CF-1	Waxy dull coloured growth with hallowing around the colonies	Broth enrichment	> 10 years	Banana field, Kannara
CF-2	White coloured flat growth with hallowing around the colonies	Soil enrichment	> 25 years	Rice field, Coimbatore
CF-3	White coloured raised colonies	Soil enrichment	> 25 years	Rice field, Coimbatore
CF-4	Dull coloured flat growth with slight hallowing around the colonies	Broth enrichment	> 5 years	Banana field, Onampalayam
CF-5	Gray coloured medium sized raised colonies	Soil enrichment	> 10 years	Banana field, Kannara
CF-6	White coloured raised growth with depressions at the centre	Soil enrichment	> 5 years	Banana field, Onampalayam
CF-7	Slimy translucent growth with slight hallowing around the colonies	Broth enrichment	> 5 years	Banana field, Coimbatore
CF-8	White coloured raised medium sized colonies	Soil enrichment	> 25 years	Rice field, Coimbatore

CF-1 (Carbofuran degrading isolate-1); CF-2 (Carbofuran degrading isolate-2); CF-3 (Carbofuran degrading isolate-3); CF-4 (Carbofuran degrading isolate-4)

CF-5 (Carbofuran degrading isolate-5); CF-6 (Carbofuran degrading isolate-6); CF-7 (Carbofuran degrading isolate-7); CF-8 (Carbofuran degrading isolate-8)

to the gradual dissolution and diffusion of the herbicides in the culture medium. This phenomenon indicated the degradative ability of the isolated strains in the present study. Chlorpyriphos degrading bacterial isolates *viz.,* CPY-1, CPY-2, CPY-3 and CPY-4 were tentatively identified as *Serretia* sp., *Pseudomonas* sp., *Klebsilla* sp. and *Acinetobacter* sp., respectively. Carbofuran degrading bacterial isolates *via.* CF-1, CF-2 and CF-7 were tentatively identified as *Enterobacter* sp., *Bacillus* sp. and *Bacillus* sp., respectively. The isolates CPY – 5, CF-3 and CF-4 were not identified after morphological and

Table 5. Morphological and biochemical characteristics of chlorpyriphos degrading bacterial isolates

Isolate	Grams Stain reaction and Shape of the cell	Nutrient Agar Plate characteristics	Fermentation			H_2S Production	NO_3 Reduction	Indole Production	MR Reaction	VP Reaction	Citrate Use	Urease Activity	Catalase Activity	Oxidase Activity	Gelatin Liquefaction	Starch Hydrolysis	Growth on Mackonkey Agar
			Glucose	Sucrose	Lactose												
CPY-1	Gram (-)ve, rods	Abundant, opaque, dull white coloured raised growth	A	A	-	-	-	+	+	-	+	-	+	-	-	-	+
CPY-2	Gram (-)ve, rods	Thin, opaque, yellow coloured wrinkled colonies	-	-	-	-	+	-	-	-	+	-	+	+	-	-	+
CPY-3	Gram (-)ve, rods	Slimy, translucent, dull coloured raised growth	A	A	-	-	+	-	+	-	+	+	+	-	-	-	+
CPY-4	Gram (-)ve, cocci	Abundant, Opaque, glistening raised growth	A	A	A	-	+	-	+	-	+	-	+	-	+	-	+
CPY-5	Gram (-)ve, rods	Abundant, translucent, dull coloured raised growth	-	-	-	-	+	-	+	-	+	+	+	-	-	+	+

A – Acid production; +ve – positive; -ve - negetive

biochemical methods of confirmation. Based on nucleotide homology and phylogenetic analysis the potential carbofuran and chlorpyriphos degrading isolates *viz.,* CF-7 and CPY- 4 were confirmed as *Bacillus subtilis* (GenBank Accession Number: EU231620) and *Acinetobacter sp.* (GenBank Accession Number: EU916708) respectively. Among the five chlorpyriphos degrading bacterial isolates, two isolates *viz.,* CPY-1 (*Serratia* sp.) and CPY-3 (*Klebsiella* sp.) were shown to belong to the family Enterobacteriaceae, one isolate (CPY-2) belonged to the genus *Pseudomonas*, and another isolate (CPY-4) belonged to the genus *Acenitobacter.* These results are in agreement with earlier reports that indicated the involvement of different species of Enterobacteriaceae in the degradation of organophosphorous pesticides like phosphonate (Lee *et al.,* 1992), glyphosate (Dick and Quinn, 1995) and chlorpyriphos (Singh *et al.,* 2004; Ghanem *et al.,* 2007; Rani *et al., 2008).* Other soil bacteria that have been isolated from the soil utilizing chlorpyriphos include *Pseudomonas disminuta, Pseudomonas putida and Bacillus subtilis* (Sethunathan and Yoshida, 1973; Rani and Lalitha Kumari, 1994, Rani *et al.,* 2008).

Table 6. Morphological and biochemical characteristics of carbofuran degrading bacterial isolates

Isolate	Gram Stain reaction and shape of the cell	Nutrient Agar Plate characteristics	Fermentation			H_2S Production	NO_3 Reduction	Indole Production	MR Reaction	VP Reaction	Citrate Use	Urease Activity	Catalase Activity	Oxidase Activity	Gelatin Liquefaction	Starch Hydrolysis	Growth on Mackonkey Agar
			Glucose	Sucrose	Lactose												
CF-1	Gram (-)ve, rods	Abundant, thick, white flat growth	AG	A	A	-	+	-	-	+	+	-	+	-	-	-	+
CF-2	Gram (+)ve, rods	Opaque, white raised and smooth growth	A	A	-	-	+	-	+	-	-	-	+	-	-	+	-
CF-3	Gram (-)ve, rods	Small, yellowish raised growth	A	-	-	-	-	+	-	+	-	-	+	-	+	+	+
CF-4	Gram (-)ve, rods	Abundant, dull coloured flat growth	A	A	-	-	+	-	-	+	-	-	+	-	-	-	+
CF-7	Gram (+)ve, rods	Abundant, waxy, circular flat growth	A	A	-	-	+	-	+	-	-	-	+	-	+	+	-

A – Acid production; G – Gas production; +ve – positive; -ve - negative

A number of bacterial isolates capable of carrying out some form of degradation of carbofuran have been isolated and reported by many workers. The several bacterial taxa includes *Pseudomonas* sp. (Felsot *et al*., 1981), *Bacillus* sp., *Arthrobacter* sp. and *Micrococcus* sp. *(*Rajagopal *et al,* 1983), *Achromobacter* sp.

(Chaudhry *et al*., 1988), *Arthrobacter* sp. (Ramanand *et al*., 1988), *Flavobacterium* (Chapalamadugu and Chaudhry, 1991), *Sphingomonas* sp. (Feng *et al*., 1997) and *Novosphingobium* sp. (Yan *et al.,* 2007). Out of the five carbofuran degrading bacteria isolated in the present study Two isolates *viz.,* CF-2 and CF-7 were shown to belong to the genus *Bacillus* and one isolate belonged to the genus *Enterobacter*. The results are in conformity with the earlier mentioned records. To conclude, chlorpyriphos degrading bacterial isolates *viz., Serretia sp., Pseudomonas sp., Klebsiella sp.* and *Acinetobacter sp.,* and Carbofuran degrading bacterial isolates *viz., Enterobacter sp., Bacillus sp.* and *Bacillus sp.,* that proved effective in degrading the pesticides for nutrient source could be cultured and used for pesticide degradation in areas were residues pose a serious problem.

References

Cappuccino, J. G. and Sherman, N. 2002. Microbiology: A Laboratory Manual. Pearson Education. New York. pp. 1-485.

Chapalamadugu, S. and Chaudhry, G. R. 1991. Hydrolysis of carbaryl by a *Pseudomonas* sp. and construction of a microbial consortium that completely metabolises carbaryl. *Appl. Environ. Microbiol.,* **57**: 744 –750.

Chaudhry G. R. and Ali, A. N. 1988. Bacterial metabolism of carbofuran. *Appl. Environ. Microbiol.,* **54**: 1414 – 1419.

Clark, C. G. and Wright, S. L. J. 1970. Detoxification of isopropyl phenyl-carbamate N-phenylcarbamate (IPC) and isopropyl N-3 chlorophenyl carbamate (CIPC) in soil and isolation of IPC-metabolizing bacteria. *Soil. Biol. Biochem.*, **1**: 19-26.

Diaz, E. 2004. Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. *Int. Microbiol.***, 7**: 173-180.

Dick, R. E. and Quinn, J. P. 1995. Glyphosate degrading isolates from environmental samples: Occurrence and pathways of degradation. *Appl. Microbiol. Biotechnol.,* **43**(8): 545-550.

Felsot, A. S., Maddox, J. V. and Bruce, W. 1981. Enhanced microbial degradation of carbofuran in soils with histories of Furadan use. *Bull. Environ. Contam. Toxicol.,* **26**: 781-788.

Feng, X., Ou, L. T. and Orgam, A. 1997. Plasmid mediated mineralization of carbofuran by *Sphingomonas* sp. Strain CF06. *Appl. Environ. Microbiol.,* **63**: 1332 – 1337.

Ghanem, I., Orfi, M. and Shamma. M. 2007. Biodegradation of chlorpyriphos by *Klebsiella sp*. isolated from an activated sludge sample of waste water treatment plant in Damascus. *Folia Microbiol.,* **54**: 423-427.

Laveglia, J. and Dahm, P. A. 1977. Degradation of organophosphorous and carbamate insecticides in the soil and by the soil microorganisms. *Ann. Rev. Entomol.,* **22:** 483-513.

Lee, K. S., Metcalf, W. W. and Wanner, B. L. 1992. Evidence for two phosphate degradative pathways in *Enterobacter aerogenes*. *J. Bacteriol.,* **174**(19): 2501-2510.

Mallick, K., Bharathi, K., Banerji, A., Shakil, N. A. and Sethunathan. N. 1999. Bacterial degradation of chlorpyriphos in pure cultures and in soil. *Bull. Environ. Contam. Toxicol.,* **62**: 48-54.

Mani, A. K., Santhi, R. and Sellamuthu, K. M. 2007. A Handbook of Laboratory Analysis. A.E. Publications, Coimbatore. pp. 72-82.

Nafeesa. M. 2009, Studies on Bacterial Degradation of Chlorpyriphos and Carbofuran Residues Using Potential Strains Isolated from the Pretreated Soils M.Sc.(Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore. pp. 74-88.

Rajagopal, B. S., Chendrayan, K., Reddy, B. R. and Sethunathan, N. 1983. Persistence of carbaryl in flooded soils and its degradation by soil enrichment cultures. *Pl. Soil,* **73**: 35.

Ramanand, K., Sharmila. M. and Sethunathan, N. 1988. Mineralization of carbofuran by a soil bacterium. *Appl. Environ. Microbiol.,* **54**: 2129–2133.

Rani, N. L. and Lalitha-Kumari, D. 1994. Degradation of methyl parathion by *Pseudomonas putida*. *Can. J. Microbiol.,* **15**: 984-987.

Rani, S., Vijaya Lakshmi, M. K., Suvarnalatha Devi, P., Jaya Madhuri, R., Aruna, S., Jyothi, K., Narasimha, G. and Venkateswarlu. K. 2008. Isolation and characterization of a chlorpyrifos degrading bacterium from agricultural soil and its growth response. *African Journal of Microbiology Research,* **2**: 026-031.

Sethunathan, N. And Yoshitha, T. 1973. A *Flavobacterium* that degrades Diazinon and parathion. *Can. J. Microbiol.,* **19**: 873-875.

Singh, B. K. and Walker, A. 2002. Degradation of chlorpyriphos, fenamiphos and chlorothalonil alone and in combination and their effects on soil microbial activity. *Environ. Toxicol. Chem.,* **21**: 2600-2605.

Singh, B. K., Walker, A., Morgan, J. A. W. and Wright, D. J. 2004. Biodegradation of chlorpyriphos by Enterobacter strain B-14 and its use in bioremediation of contaminated soil. *Appl. Environ. Microbiol.,* **70**: 4855-4863.

Slaoui, M., Uhssine, M., Berny, E. and M. Elyachioui. 2007. Biodegradation of the Carbofuran by a fungus isolated from treated soil. *Afr. J. Biotechnol.,* **6** (4): 419-423.

Yan, Q. X., Hong, Q., Han, P., Dong, X. J., Shen, Y. J. and Li, S. P. 2007. Isolation and characterisation of carbofuran-degrading strain *Novosphingobium* sp. FND-3. *FEMS Microbiol. Lett,.* **271**: 207-213.

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