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Biodegradation of the fungicide carbendazim by bacteria from *Coriandrum sativum* L. rhizosphere

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ABSTRACT. The biocidal agrochemicals commonly used in agriculture can remain in the soil, affecting the environmental conditions and causing serious risks to health. Knowing that soil microorganisms, especially those from the rhizosphere, can degrade environmental xenobiotics, it was evaluated the potential of bacteria isolated from *Coriandrum sativum* L. rhizosphere to biodegrade carbendazim (MBC), a fungicide extensively used by agriculturists from rural farming communities in Manaus, Amazonas. Cultures carried out in medium containing carbendazim as a sole carbon source enabled the isolation of 80 bacteria, in the established conditions. Assays to determine degradation potential allowed the selection of the two elite isolates identified as *Stenotrophomonas* sp. and *Ochrobactrum* sp. Quantitative assays with each strain individually or in consortium, were carried out using minimal salt medium added with carbendazim (250 μg mL^{-1}) and incubated at 30°C, under agitation (125 rpm) for 21 days. Samples used in the biodegradation test were HPLC analyzed for final fungicide quantitation. The *Stenotrophomonas* sp. strain was more efficient (68.9%) to degrade carbendazim and showed no toxicity in tests with *Artemia salina*.

Keywords: Amazon soil, fungicide, coriander, carbendazim-degrading bacteria, *Stenotrophomonas*, xenobiotics.

Biodegradação do agrotóxico carbendazim por bactérias da rizosfera de *Coriandrum sativum* L.

RESUMO. Agrotóxicos são comumente utilizados na produção agrícola, podendo persistir no solo, afetar a qualidade do ambiente e causar sérios riscos à saúde. Sabendo-se que micro-organismos do solo, principalmente aqueles da rizosfera, podem degradar produtos xenobióticos avaliou-se o potencial de bactérias isoladas da rizosfera de *Coriandrum sativum* L. em degradar carbendazim, um fungicida usado extensivamente em comunidades de agricultores rurais em Manaus, Amazonas. Procedimentos de cultivo em meio, contendo carbendazim como única fonte de carbono, mostraram que 80 bactérias cresceram nas condições estabelecidas. Ensaios de eficiência de degradação permitiram a seleção dos dois melhores isolados que foram identificados como *Stenotrophomonas* sp. e *Ochrobactrum* sp. Os ensaios quantitativos, com cada cepa individualmente e com as duas em consórcio, foram conduzidos em meio mínimo contendo sais, acrescido de carbendazim (250 μg mL^{-1}) e incubados a 30°C, 125 rpm, por 21 dias. A quantificação final do fungicida nas amostras do ensaio de biodegradação foi realizada em HPLC. A linhagem *Stenotrophomonas* sp. apresentou maior eficiência, degradando 68,9% do total de carbendazim e não apresentou toxicidade nos testes realizados com *Artemia salina*.

Palavras-chave: Solo da Amazônia, fungicida, coentro, degradação de carbendazim, *Stenotrophomonas*, xenobióticos.

Introduction

The use of benzimidazoles, a group of organic fungicides with systemic action started in the 1960s, became a milestone in the history of the development of fungicides. They are indicated in soil and foliar applications, and in seed treatment (Silva, Melo, Maia, & Abakerli, 1999). Among this group of fungicides there is carbendazim (MBC), a commonly used agrochemical effective against a wide variety of diseases such as those caused by Ascomycetes, Basidiomycetes and Mitosporic fungi in fruit and vegetable crops (Roberts & Hutson, 1999; Boudina, Emmelin, Baaliouame, Grenier-Loustalot, & Chovelon, 2003). *Agência Nacional de Vigilância Sanitária* [ANVISA] (2003) classified the effects of MBC as moderately toxic (class III), it was considered very toxic to the aquatic environment (Palanikumar, Kumaraguru, Ramakritinan, & Anand, 2014). Moreover, in the soil ecosystem there

is a high environmental risk, what may reduce the function and alter the soil microbiota composition (Bueno, Meyer, & Souza, 2003; Wang, Huang, Chen, & Yen, 2009).

MBC residues can be absorbed by plant crops and transferred along the food chain to humans (Singhal, Bagga, Kumar, & Chauhan, 2003) and may cause humoral disorders and cellular immunity (Klucinski, Kossmann, Tustanowski, Friedek, & Kaminska-Kolodziej, 2001). Studies with mice have shown that long periods of exposure to MBC can lead to decreased survival rate, promote hematological, biochemical and histopathological alterations in adrenal glands, thyroid and liver (Yu, Guo, Xie, Liu, & Wang, 2009), cause chromosomal aberrations (Kirsch-Volders, Vanhauwaert, Eichenlaub-Ritter, & Decordier, 2003) and can cause endocrine disruption of male reproductive system (Hess & Nakai, 2000).

Carbendazim may be slowly degraded via chemical and physical processes (Mazellier, Leroy, De Laat, & Legube, 2003) and also by the rhizosphere microbial community (Roque & Melo, 2000). Fungicide mineralization can be increased by rhizospheric bacterial activity or natural selection of more efficient microorganisms (Hsu & Bartha, 1979). In cases involving biodegradation, this process can be attributed to the efficacy of fungi (Silva et al., 1999; Melo, Silva, & Faull, 2010), actinomycetes (Xu et al., 2007; Sun et al., 2014) and bacteria (Fang et al., 2010; Ji, Lijun, Shibin, & Ron, 2016).

The increased demand for food and the introduction of non-native cultures and therefore more susceptible to pests, imposed to the agriculturists in Amazon a more intensive use of pesticides (Römbke, Waichman, & Garcia, 2008), mainly insecticides, herbicides and fungicides (Ecobichon, 2001).

Usually, sandy soils present low water holding capacity and low organic matter content (Oliveira, 2010). According to Santos et al. (2017) the soil at Nova Esperança, a rural farming community located within the limit of Manaus, AM, Brazil, where the *Coriandrum sativum* (coriander) rhizosphere soil samples were collected, presents a sandy texture and at 10-20 cm depth is considered fertile. Moreover, this soil exhibit slow alkalinity, with pH values within the range of 7.5 and 7.6, low ratios of potassium, magnesium and boron, and absence of aluminum.

However, *C. sativum* rhizosphere soil samples were collected from a cultivation area used for growing vegetables, where the replacement of organic matter is continuous, providing sufficient nutrient to supply the needs of the plants and to maintain a microbial community in the rhizosphere. Multi-residue analysis of the soil samples investigated in this study lead to the detection of 0.189 μg $kg^{-1}$of MBC fungicide.

So, the aim of this paper was to characterize the bacterial rhizosphere community of *C. sativum* from cultivated areas in Nova Esperança (Manaus, AM, Brazil), and evaluate the biodegradation of carbendazim (MBC) by the isolated bacteria.

Material and methods

Soil Collection

Soil samples were collected in Nova Esperança, a rural farming community located within the limits of Manaus, AM, Brazil, in the east area of the city, following ISO/FDIS 10381-1: 2001 guidelines, which consists of a sampling in X shape design, in the following geographical coordinates: sample 1: S3º00'56".5 W59º55'14.0"; sample 2: S3º00'56.9" W59º55'13.7"; sample 3: S3º00'57.7" W59º55'13.5"; sample 4: S3º00'57.7" W59º55'14.2"; sample 5: S3º00'57.3" W59º55'14.1".

Selective isolation of bacteria

Portions (6.6 g) from 5 samples of soil were homogenized totalizing 33 g of a composite soil sample, which was inoculated in a 2 L Erlenmeyer flask containing 500 mL of minimal salts medium - MMS (K_2HPO_4 (2.78 g L^{-1}), (NH_4) $2SO_4$ (1.0 g L^{-1}), $MgCl_2.6H_2O$ (0.2 g L^{-1}), NaCl (0.1 g L^{-1}), $FeCl_3.6H_2O$ (0.02 g L^{-1}), $CaCl_2$ (0.01 g L^{-1}) - to 1 L) and carbendazim - MBC (250 μg mL^{-1}). A negative control was prepared containing only MMS (500 mL) and carbendazim (250 μg mL^{-1}), without the soil sample. The flasks were incubated at 28°C under agitation (125 rpm) for seven days for rigorous selection process. After that period, aliquots of 100 mL were removed from each flask and inoculated again in 400 mL of MMS and MBC for more seven days, under the same conditions. This procedure was repeated for two more times. After the last incubation period, aliquots of 1 mL from each flask were submitted to serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}), using 0.85% saline solution. Aliquots (100 μL) from each dilution were plated on Petri dishes containing MMS agar + MBC (250 μg mL^{-1}). The experiments were carried out in triplicate. With the aid of a Drigalski handle, the cells were uniformly distributed on the plates which were incubated in BOD at 28°C for ten days to isolate bacteria and fungi. The bacteria which grew were transferred to test tubes containing 5 mL Luria Bertani - LB agar (Himedia) culture medium.

Molecular analysis

The bacteria were identified following method described by Mota, Back-Brito, and Nóbrega (2009). Amplification of 16S rDNA was performed by PCR using 095F (5' - GTG CCA GCM GCN GGC G - 3') and 094R (5' - GGY TAC CTT GTT ACG ACT T - 3') primers. The sequencing was carried out on an ABI 3500xL automated sequencer (Applied Biosystems®). Sequences alignments were edited using MEGA6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) and combined clustering/similarity methods (BLASTn/neighbor joining) were used for identification and comparison with GenBank database sequences.

Inoculum preparation for biodegradation assays

The bacterial inoculum was prepared from stock cultures LB kept at -20°C, which were transferred to plates containing LB and incubated at 30°C for 24 hours. With the aid of a platinum handle, an aliquot containing approximately 10^6 CFU mL^{-1} bacterial suspension was transferred to 15 mL Falcon type tubes containing 3 mL of LB broth and were incubated at 30°C and under agitation (250 rpm) for 4 hours. The resultant culture was centrifuged (1792 rcf) for 10 minutes. The supernatant was discarded and the cell pellet suspended with MMS. Bacterial biomass was diluted in MMS to obtain O.D. equal to -1 absorbance at 610 nm, which corresponds to a population of 10^9 CFU mL^{-1}. In the biodegradation tests were used aliquots (1 mL) of bacterial inoculum suspension. In the consortium of selected bacteria, were utilized aliquots (500 μL) of each bacterial isolate.

Selection of microorganisms and carbendazim biodegradation assays

Degradation tests were carried out with the two finest strains selected after preliminary tests for evaluation of bacterial growth measured by optical density after 72 hours of culture. Concurrently, the degradation was quantified by high-performance liquid chromatography - HPLC. The biodegradation experiments with each isolate or with a consortium of both bacteria were performed in triplicate. Aliquots (1 mL) of the bacterial suspension (D.O. 1) were inoculated in 125 mL Erlenmeyer flask containing MMS (50 mL) and 250 mg L^{-1} MBC (97% purity, Sigma-Aldrich) diluted in dimethylsulfoxide (DMSO) and incubated at 30°C under agitation (125 rpm) for 21 days. The positive control group (LB and bacteria) was used to monitor the bacterium viability.

Extraction of carbendazim

The extraction of MBC was performed using ethyl acetate. To each Erlenmeyer flask, 50 mL of ethyl acetate was added. The material was transferred to a 125 mL separatory funnel and subjected to vigorous agitation for 1 minute. The organic phase was collected and the aqueous phase was extracted twice more, by the addition of 50 mL of ethyl acetate. The end of the extraction was monitored by thin layer chromatography (TLC). In the collected organic phase, anhydrous sodium sulfate was added and after filtration the samples were subjected to vacuum rotary evaporator at 40°C until reduction of the volume of the sample to approximately 10 mL. The sample was transferred to 10 mL volumetric flask containing 10 μg mL^{-1} of an internal standard solution. The volume of 1 μL of the sample was injected into HPLC.

Biodegradation data analysis

The internal standard and surrogate solutions were added with phenanthrene-d10 (phe-d10) and naphthalene d8 (nap-d8) with a purity ≥ 98% (Sigma-Aldrich, 98%), solubilized in HPLC grade hexane. The internal standard (nap-d8) was inserted in the samples diluted in a volumetric flask before injected into HPLC. The surrogate (phe-d10) was inserted in Erlenmeyer flasks before starting the extraction process. The use of these patterns is needed to recognize possible losses during the extraction process. The chromatographic area of the analyte was corrected by extraction recovery rate, which was obtained by the formula: [(*CAs*/*CAis*] x 100, where *CAis* corresponds to the chromatographic area of the internal standard and the *CAs* for the chromatographic area of the *surrogate*. The MBC concentration was obtained by a calibration curve using different MBC concentrations: 100, 200, 300, 500, 700, 900 and 1200 μg mL^{-1}. The percentage of MBC degradation was calculated by the formula: [(*CC* - *CE*)/*CC*] x 100, where *CC* is the MBC concentration in the control group and *CE* the remaining concentration of MBC from experiment. Finally, it was calculated the average and the standard deviation of the biodegradation rate in order to obtain greater accuracy of MBC concentration.

HPLC analysis

HPLC analyses of MBC were performed in a Shimadzu (CBM–20A) Prominence® UFLC system, coupled with high-pressure binary pump (LC-20AD); auto injector (SIL-20AHT), detector UV-Vis (SPD-20A); column oven heating; CTO-

20A. The column used was Phenomenex Luna C18 (250 mm x 4.6 mm, 5u). The Solvent A: ultrapure water/0.1% formic acid and the Solvent B: acetonitrile grade HPLC/0.1% formic acid. Flow: 1.0 mL min^{-1}. Gradient: 0-2 min, linear gradient from 60 to 85% B; 2-8 min, isocratic at 85% B; 8-9 min, linear gradient from 85 to 60% B. Wavelength set at 285 nm. Retention times at 1.9; 5.9 and 7.9 min for MBC, naphthalene and phenantrene, respectively.

Toxicity test

Following the biodegradation assays, a toxicity test was carried out with *Artemia salina* in triplicates for both strains and the consortium as described by Atayde, Carneiro, Martins, and Palheta (2011). Determination of lethal concentration (LC_{50}) values was performed as described by Harwig & Scott (1971) in triplicate only for extracts that showed toxicity.

Results and discussion

Isolation and molecular analysis of bacteria

Coriandrum sativum rhizosphere soil inoculated in selective medium containing carbendazim as a sole carbon source enabled the isolation of 80 bacteria. It was possible to identify 60 strains and 52 different possible species. Table 1 shows the 20 most common bacteria identified by rDNA sequencing. The predominant genera were *Bacillus* sp., *Ochrobactrum* sp., *Enterobacter* sp. and *Stenotrophomonas* sp. Among the identified isolates, the genus *Bacillus* was the most representative, with 28% frequency. From an area adjacent to the area investigated in this study, which had been treated with the fungicide Mancozeb, Rebière (2015) isolated 23 bacteria from rhizosphere soil of *Allium fistulosum* L. (Welsh onion) crops of which 81.81% were identified as *Bacillus* sp. Among 23 bacteria isolated from rhizosphere soil collected in China, Hu, Zhao, Liu, Song, and You (2013) identified the genus *Ochrobactrum* which is able to grow in MMS medium added with the insecticide Imidacloprid (50 μg mL^{-1}).

After 72 hours of culture in medium containing carbendazim as sole carbon source, two bacterial isolates were selected (*Stenotrophomonas* sp. and *Ochrobactrum* sp.) for qualitative and quantitative biodegradation tests. The two selected genera had their sequence annotation submitted to the GenBank-NCBI and were deposited as *Stenotrophomonas* sp. (accession number KX376306) and *Ochrobactrum* sp. (accession number KX376307).

The genus *Stenotrophomonas* sp. is found in harsh environments such as Antarctica (Vazquez, Ruberto, & Mac Cormack, 2005) and can produce bioactive substances, such as laccase enzyme (Galai, Limam, & Marzouki, 2009). Some bacterial strains can degrade PAHs (Zafra, Absalón, Cuevas, & Cortés-Espinosa, 2014), as well as the fungicide Chlorothalonil (Zhang et al., 2014). *Stenotrophomonas* sp. and *Ochrobactrum* sp. can degrade Chlorpyrifos (Talwar, Mulla, & Ninnekar, 2014; Deng et al., 2015.), but there is no report on the efficiency of *Stenotrophomonas* sp. and *Ochrobactrum* sp. to degrade carbendazim. Therefore, it is important further studies to validate the potential of those microorganisms for degrading carbendazim in Amazonian soils.

Table 1. The twenty major bacterial isolates able to grow in selective medium containing carbendazim as sole carbon source.

Isolates	Species	ID	GenBank
JS19	*Bacillus methylotrophicus*	99%	KF376347.1
JS72	*Bacillus* sp.	99%	KF554081.1
JS75	*Bacillus pumilus*	98%	KC771041.1
JS4	*Stenotrophomonas maltophilia*	99%	LN623619.1
JS32	*Stenotrophomonas* sp.	98%	FJ765513.1
JS1	*Ochrobactrum pseudointermedium*	96%	JQ337956.1
JS3	*Ochrobactrum intermedium*	98%	JN613288.1
JS69	*Ochrobactrum* sp.	98%	KM975676.1
JS73	*Ochrobactrum* sp.	99%	JX535021.1
JS5	*Enterobacter* sp.	99%	KF420155.1
JS8	*Enterobacter* sp.	99%	KM870909.1
JS77	*Klebsiella* sp.	96%	EF647622.1
JS22	*Staphylococcus saprophyticus*	96%	JQ229688.1
JS41	*Micrococcus luteus*	98%	CP007437.1
JS42	*Agrobacterium* sp.	96%	KJ184969.1
JS44	*Pseudomonas nitroreducens*	96%	KC355311.1
JS57	*Brevibacillus* sp.	98%	AJ586388,1
JS58	*Paenibacillus* sp.	95%	KJ769171.1
JS60	*Staphylococcus nepalensis*	98%	JX254661.1
JS68	*Paenochrobium glaciei*	97%	NR112750.1

The growth of the bacterial isolates, cultured alone or in consortium, for 21 days in culture medium containing carbendazim, was determined by optical density and chromatographic methods.

The *Stenotrophomonas* sp. and the consortium of both *Stenotrophomonas* sp. and *Ochrobactrum* sp. when cultured at a concentration of 250 μg mL^{-1} carbendazim showed the best growth rates (Figure 1). Zhang et al. (2014) evaluated the ability of *Stenotrophomonas* sp. strain to degrade the fungicide chlorothalonil cultured in minimal salt medium added with chlorothalonil (20 μg mL^{-1}) for 7 days. *Stenotrophomonas* sp. optical density ranged 0.17.

The obtained results indicate that both *Stenotrophomonas* sp. and *Ochrobactrum* sp. can degrade carbendazim without supplementary sources of carbon. Fang et al. (2010), Zhang et al. (2013), and Salunkhe et al. (2014) showed that bacterial strains used in biodegradation tests can be isolated using carbendazim as the only source of carbon. According to Fang et al. (2010), such characteristics are required when isolating microorganisms to be used for bioremediation purposes.

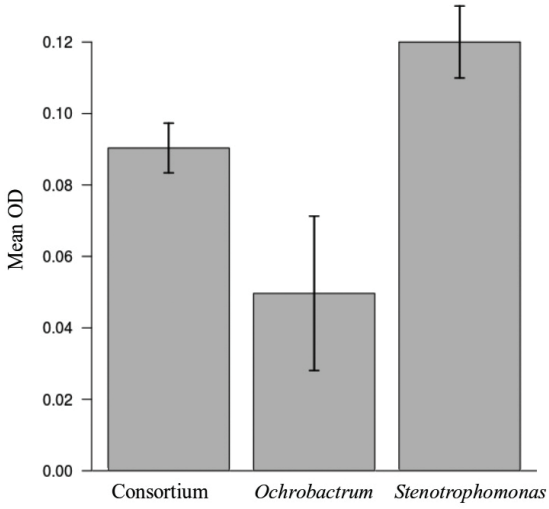

Figure 1. Optical density of culture medium containing *Ochrobactrum* sp. or *Stenotrophomonas* sp. bacterial isolates from *Coriandrum sativum* L. rhizosphere cultured for 21 days at 30°C under agitation (125 rpm) separately or in consortium. Error bars represent standard errors of the means.

Carbendazim biodegradation analyzed by HPLC

HPLC analysis of extracts from *Stenotrophomonas* sp. cultures indicated the higher potential (68.9%) of this bacterial isolate to degrade carbendazim, if compared to the isolate *Ochrobactrum* sp. cultured separately or in consortium with *Stenotrophomonas* sp. (Figure 2).

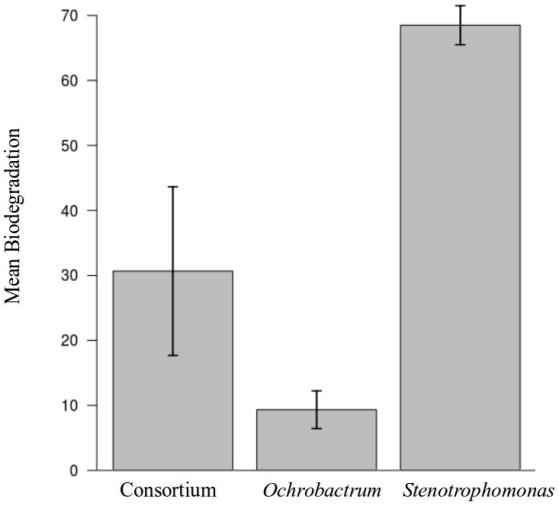

Figure 2. Biodegradation of carbendazim in 21 days by bacteria isolated from *Coriandrum sativum* L. rhizosphere. Error bars represent standard errors of the means.

Several bacteria efficient in degrading MBC have been cited in the literature, such as *Pseudomonas* sp. (Xiao et al., 2013), *Bacillus subtilis* TL-171 (Salunkhe et al., 2014), and *Brevibacillus borstelensis* (Arya & Sharma, 2015). Salunkhe et al. (2014) reported that four strains of *B. subtilis* individually degraded from 75.7 to 95.2% MBC in a concentration of 10 μg mL^{-1} after 15 days of incubation. The concentration of MBC used to validate the degradation potential by the bacterial isolates *Stenotrophomonas* sp. and *Ochrobactrum* sp. was 25% higher than that used by Salunkhe et al. (2014).

The lowest degradation percentage showed by the consortium of both bacterial isolates may have occurred due to competition for nutrients caused by the co-cultivation of isolates of different genera. However, Arya & Sharma (2015) investigating the degradation ability of bacteria alone or in consortium found a significant reduction of MBC caused by bacteria in consortium. Luo et al. (2009) isolated three marine bacterial lineages: *Ochrobactrum* sp., *Stenotrophomonas* sp., and *Pseudomonas* sp. to degrade benzopyrene an aromatic polycyclic hydrocarbon of high molecular weight. Faster degradation was observed when the isolates were co-cultured in consortium.

The obtained results indicate that the isolate *Stenotrophomonas* sp. cultured alone shows greater potential to degrade carbendazim if compared to *Ochrobactrum* sp. MBC degradation potential, no matter if cultured alone or in consortium with *Stenotrophomonas* sp.

Toxicity tests using *Artemia salina*

Values obtained in toxicity tests carried out with *A. salina* (Table 2) ranged 0-0.7% using *Stenotrophomonas* sp. extract and the consortium of both isolates. Harwig & Scott (1971) established criteria for the extract toxicity classification using only the number of microcrustaceans and considered as non-toxic (NT), mortality less than 0.9%. However, *Ochrobactrum* sp. extracts showed slight toxicity (LT).

Table 2. Toxicity tests using *Artemia salina*

Positive Control	Bacterial Extracts	(M%)
0.2%	Consortium of both isolates	0%
0%	*Ochrobactrum* sp.	38%
0.7%	*Stenotrophomonas* sp.	0%

M% - mortality percentage

From the result with the *Ochrobactrum* sp., culture extracts of this lineage were tested in *A. salina* at concentrations of 50, 25, 13, 7 and 3% to determine the lethal concentration (LC50), median lethal dose values indicated that extracts from this isolate are not toxic in all tested concentrations.

Conclusion

The *Stenotrophomonas* sp. strain was able to degrade 68.9% of carbendazim in a mineral culture medium containing 250 μg mL^{-1} of the fungicide. In

addition, the resulting extract showed no toxicity in tests with *A. salina*. These results enhance the importance of further studies with this strain to validate its application on field.

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References

Agência Nacional de Vigilância Sanitária [ANVISA] (2003, 2 de setembro). Resolução RE nº 165, de 29 de agosto de 2003. *Diário Oficial de União*, Brasilia, DF, n. 169, Seção 1, pág. 48.

Arya, R., & Sharma, A. K. (2015). Bioremediation of carbendazim, a benzimidazole fungicide using *Brevibacillus borstelensis* and *Streptomyces albogriseolus* together. *Current Pharmaceutical Biotechnology, 17*(2), 185-189.

Atayde, H. M., Carneiro, A. L. B., Martins, M. S., & Palheta, R. A. (2011). Bioensaios de toxicidade com espécies de *Artemia*. In M. F. S. Teixeira, T. A. Silva, R. A. Palheta, A. L. B. Carneiro, & H. M. Atayde (Eds.), *Fungos da Amazônia: Uma riqueza inexplorada (aplicações biotecnológicas)* (p. 190-207). Manaus, AM: Editora da Universidade Federal do Amazonas.

Boudina, A., Emmelin, C., Baaliouamer, A., Grenier-Loustalot, M. F., & Chovelon, J. M. (2003). Photochemical behaviour of carbendazim in aqueous solution. *Chemosphere, 50*(5), 649-655.

Bueno, C. J., Meyer, M. C., & Souza, N. L. (2003). Efeito de fungicidas na sobrevivência de *Bradyrhizobium japonicum* (Semia 5019 e Semia 5079) e na nodulação da soja. *Acta Scientiarum. Agronomy, 25*(1), 231-235.

Deng, S., Chen, Y., Wang, D., Shi, T., Wu, X., Ma, X., ... Hua, R. (2015). Rapid biodegradation of organophosphorus pesticides by *Stenotrophomonas* sp. G1. *Journal of Hazardous Materials*, *297*(1), 17-24.

Ecobichon, D. J. (2001). Pesticide use in developing countries. *Toxicology, 160*(1-3), 27-33.

Fang, H., Wang, Y., Gao, C., Yan, H., Dong, B., & Yu, Y. (2010). Isolation and characterization of *Pseudomonas* sp. CBW capable of degrading carbendazim. *Biodegradation, 21*(6), 939-946.

Galai, S., Limam, F., & Marzouki, M. N. (2009). A new *Stenotrophomonas maltophilia* strain producing laccase. Use in decolorization of synthetics dyes. *Applied Biochemistry and Biotechnology, 158*(2), 416-431.

Harwig, J., & Scott, P. M. (1971). Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Applied Microbiology*, *21*(6), 1011-1016.

Hess, R. A., & Nakai, M. (2000). Histopathology of the male reproductive system induced by the fungicide benomyl. *Histology and Histopathology, 15*(1), 207-224.

Hsu, T. S., & Bartha, R. (1979). Accelerated mineralization of two organophosphate insecticides in the rhizosphere. *Applied and Environmental Microbiology, 37*(1), 36-41.

Hu, G., Zhao, Y., Liu, B., Song, F., & You, M. (2013). Isolation of an indigenous imidacloprid-degrading bacterium and imidacloprid bioremediation under simulated *in situ* and *ex situ* conditions. *Journal of Microbiology and Biotechnology, 23*(11), 1617-1626.

Ji, L., Lijun, R., Shibin, H., & Ron, C. H. (2016). Isolation and characterization of the carbendazim-degrading strain Djl-5B. *Nature Environment and Pollution Technology, 15*(1), 97-102.

Kirsch-Volders, M., Vanhauwaert, A., Eichenlaub-Ritter, U., & Decordier, I. (2003). Indirect mechanisms of genotoxicity. *Toxicology Letters*, *140-141*, 63-74.

Klucinski, P., Kossmann, S., Tustanowski, J., Friedek, D., & Kaminska-Kolodziej, B. (2001). Humoral and cellular immunity rates in chemical plant workers producing dust pesticides. *Medical Science Monitor,* 7(6), 1270-1274.

Luo, Y. R., Tian, Y., Huang, X., Yan, C. L., Hong, H. S., Lin, G. H., & Zheng, T. L. (2009). Analysis of community structure of a microbial consortium capable of degrading benzo(a)pyrene by DGGE. *Marine Pollution Bulletin, 58*(8), 1159-1163.

Mazellier, P., Leroy, É., De Laat, J., & Legube, B. (2003). Degradation of carbendazim by UV/H_2O_2 investigated by kinetic modelling. *Environmental Chemistry Letters, 1*(1), 68-72.

Melo, I. S., Silva, C. M. M. S., & Faull, J. L. (2010). Isolation and characterization of carbendazim degrading *Trichoderma harzianum* rifai mutants. *Pesticidas: Revista de Ecotoxicologia e Meio Ambiente, 20*(1), 1-6.

Mota, A. J., Back-Brito, G. N., & Nóbrega, F. G. (2009). A practical and rapid microplate method for yeast genomic DNA extraction. *Mycoses, 52*(1), 94.

Oliveira, D. L. (2010). Solos uma questão de sustentabilidade. *Gestão e Tecnologia, 2*(3), 30-42.

Palanikumar, L., Kumaraguru, A. K., Ramakritinan, C. M., & Anand, M. (2014). Toxicity, biochemical and clastogenic response of chlorpyrifos and carbendazim in milkfish *Chanos chanos*. *International Journal of Environmental Science and Technology, 11*(3), 765-774.

Rebière, C. (2015). Identificação molecular e fenotípica de bactérias de solo rizosférico com tolerância ao fungicida Mancozeb, em Manaus, Estado do Amazonas, Brasil. *Revista Pan-Amazônica de Saúde, 6*(2), 37-43.

Roberts, T. R., & Hutson, D. H. (1999). Benzimidazoles. In T. R. Roberts, D. H. Hutson, P. W. Lee, P. H. Nicholls, & J. R. Plimmer (Eds.), *Metabolic pathways of agrochemicals: Part 2: Insecticides and fungicides* (p. 1105-1112). Cambridge: UK: Royal Society of Chemistry.

Römbke, J., Waichman, A. V., & Garcia, M. V. B. (2008). Risk assessment of pesticides for soils of the Central Amazon, Brazil: Comparing outcomes with temperate

and tropical data. *Integrated Environmental Assessment and Management, 4*(1), 94–104.

Roque, M. R. A., & Melo, I. S. (2000). Isolamento e caracterização de bactérias degradadoras do herbicida diuron. *Scientia Agricola, 57*(4), 723-728.

Salunkhe, V. P., Sawant, I. S., Banerjee, K., Wadkar, P. N., Sawant, S. D., & Hingmire, S. A. (2014). Kinetics of degradation of carbendazim by *B. subtilis* strains: Possibility of *in situ* detoxification. *Environmental Monitoring and Assessment*, *186*(12), 8599-8610.

Santos, J. C., Lima, J. M. S, Teles, Y. V, Araújo, S. P., Costa Neto, P. Q., Mota, A. J., & Pereira, J. O. (2017). Caracterização da microbiota da rizosfera resistentes ao agrotóxico carbendazim em culturas de coentro na Comunidade Nova Esperança em Manaus/AM. In L. A. Oliveira, O. C. Fernandes, M. A. Jesus, J. L. S. Bentes, S. L. Andrade, A. Q. L. Souza, & C. Santos (Eds), *Diversidade microbiana da Amazônia* (p. 212-220). Manaus, AM: INPA.

Silva, C. M. M. S., Melo, I. S., Maia, A. H. N., & Abakerli, R. B. (1999). Isolamento de fungos degradadores de carbendazim. *Pesquisa Agropecuária Brasileira, 34*(7), 1255-1264.

Singhal, L. K., Bagga, S., Kumar, R., & Chauhan, R. S. (2003). Down regulation of humoral immunity in chickens dueto carbendazim. *Toxicology in Vitro, 17*(5-6), 687-692.

Sun, L. N., Zhang, J., Gong, F. F., Wang, X., Hu, G., Li, S. P., & Hong, Q. (2014). *Nocardioides soli* sp. nov., a carbendazim-degrading bacterium isolated from soil under the long-term application of carbendazim. *International Journal of Systematic and Evolutionary Microbiology, 64*(6), 2047-2052.

Talwar, M. P., Mulla, S. I., & Ninnekar, H. Z. (2014). Biodegradation of organophosphate pesticide quinalphos by *Ochrobactrum* sp. Strain HZM. *Journal of Applied Microbiology, 117*(5), 1283-1292.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*(12), 2725-2729.

Vazquez, S., Ruberto, L., & Mac Cormack, W. (2005). Properties of extracellular proteases from three psychro tolerant *Stenotrophomonas maltophilia* isolated from Antarctic soil. *Polar Biology, 28*(4), 319-325.

Wang, Y. S., Huang, Y. J., Chen, W. C., & Yen, J. H. (2009). Effect of carbendazim and pencycuron on soil bacterial community. *Journal of Hazardous Materials, 172*(1), 84-91.

Xiao, W., Wang, H., Li, T., Zhu, Z., Zhang, J., He, Z., & Yang, X. (2013). Bioremediation of Cd and carbendazim co-contaminated soil by Cd-hyper accumulator *Sedum alfredii* associated with carbendazim-degrading bacterial strains. *Environmental Science and Pollution Research*, *20*(1), 380-389.

Xu, J. L., He, J., Wang, Z. C., Wang, K., Li, W. J., Tang, S. K., & Li, S. P. (2007). *Rhodococcus qingshengii* sp. nov., a carbendazim-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology, 57*(1), 2754-2757.

Yu, G., Guo, Q., Xie, L., Liu, Y., & Wang, X. (2009). Effects of subchronic exposure to carbendazim on spermatogenesis and fertility in male rats. *Toxicology and Industrial Health, 25*(1), 41-47.

Zafra, G., Absalón, Á. E., Cuevas, M. D. C., & Cortés-Espinosa, D. V. (2014). Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soils. *Water Air Soil Pollut, 225*(2), 1826.

Zhang, M. Y., Teng, Y., Zhu, Y., Wang, J., Luo, Y. M., Christie, P., Li, Z.-G., & Udeigwe, T. K. (2014). Isolation and characterization of chlorothalonil-degrading bacterial strain H4 and its potential for remediation of contaminated soil. *Pedosphere, 24*(6), 799-807.

Zhang, X., Huang, Y., Harvey, P. R., Li, H., Ren, Y., Li, J., … Yang, H. (2013). Isolation and characterization of carbendazim-degrading *Rhodococcus erythropolis* djl-11. *Plos One, 8*(10), e74810.

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