

ISOLATION AND CHARACTERIZATION OF FUNGICIDE DEGRADING BACTERIA FROM CONTAMINATED AGRICULTURE SOIL

Sanjana Bhagat*

Department of Biotechnology, Govt Nagarjuna PG College of Science, Raipur Chhattisgarh.

Article Received: 21 March 2023 | Article Revised: 10 April 2023 | Article Accepted: 30 April 2023

*Corresponding Author: Sanjana Bhagat

Department of Biotechnology, Govt Nagarjuna PG College of Science, Raipur Chhattisgarh.

DOI: <https://doi.org/10.5281/zenodo.23417598>

How to cite this Article: Sanjana Bhagat (2023) ISOLATION AND CHARACTERIZATION OF FUNGICIDE DEGRADING BACTERIA FROM CONTAMINATED AGRICULTURE SOIL. World Journal of Pharmaceutical Science and Research, 2(2), 58-66.



Copyright © 2023 Sanjana Bhagat | World Journal of Pharmaceutical Science and Research.

This work is licensed under creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0).

ABSTRACT

There are a number of health hazards associated with use of pesticides like Hexaconazole and carbendazim the residues of which were detected in vast number of samples at different areas near to their application. In this study, we emphasized upon the use of micro-organisms for the remediation of carbendazim and hexaconazole and tried to address the mechanism of pesticide degradation despite having limited information available in literature. The concern about harmful effects of pesticides raises further the need to develop better remediation technologies by using and discovering efficient carbendazim and Hexaconazole degrading microorganisms which could eradicate or reduce the contamination of fruits, vegetables, crops, soil, ground water and environment caused by these pesticides and hence preventing these chemicals from becoming health risk to human and animal race.

KEYWORDS: Pesticides, Bioremediation, fungicide, xenobiotic, minimum inhibitory concentration.

INTRODUCTION

The rapidly growing industrialization along with an increasing population has resulted in the accumulation of a wide variety of chemicals. Thus, the frequency and widespread use of man-made "xenobiotic" chemicals has led to a remarkable effort to implement new technologies to reduce or eliminate these contaminants from the environment. Commonly used pollution treatment methods (e.g. land-filling, recycling, pyrolysis and incineration) for the remediation of contaminated sites have also had adverse effects on the environment, which can lead to the formation of toxic intermediates. Furthermore, these methods are more expensive and sometimes difficult to execute, especially in extensive agricultural areas, as for instance pesticides. One promising treatment method is to exploit the ability of microorganisms to remove pollutants from contaminated sites, an alternative treatment strategy that is effective, minimally hazardous, economical, versatile and environment-friendly, is the process known as bioremediation. Thereafter, it was discovered that microbes have the ability to transform and/or degrade xenobiotic; scientists have been

exploring the microbial diversity, particularly of contaminated areas in search of organisms that can degrade a wide range of pollutants. Hence, biotransformation of organic contaminants in the natural environment has been extensively studied to understand microbial ecology, physiology and evolution due to their bioremediation potential.^[1-5]

Thus, microorganisms provide a potential wealth in biodegradation. The ability of these organisms to reduce the concentration of xenobiotic is directly linked to their long-term adaptation to environments where these compounds exist. Moreover, genetic engineering may be used to enhance the performance of such microorganisms that have the preferred properties, essential for biodegradation. About 30% of agricultural produce is lost due to pests. Hence, the use of pesticides has become indispensable in agriculture. The abusive use of pesticides for pest control has been widely used in agriculture. However, the indiscriminate use of pesticides has inflicted serious harm and problems to humans as well as to the biodiversity. The problem of environmental contamination by pesticides goes beyond the locality where it is used. The agricultural pesticides that are exhaustively applied to the land surface travel long distances and can move downward until reaching the water table at detectable concentrations, reaching aquatic environments at significantly longer distances. Therefore, the fate of pesticides is often uncertain; they can contaminate other areas that are distant from where they were originally used. Thus, decontaminating pesticide-polluted areas is a very complex task.^[6-8]

The state of Chhattisgarh is agriculture state and more developing at very fast rate and has a number of chemical compounds such as fungicide, herbicide, insecticide, fertilizers and other compounds used for the improvement of good quality and quantity of agriculture product. Continuously use of this compound cause the harmful to the biotic and abiotic factors and play a major role as environment pollutants.

Hexaconazole and Carbendazim are widely used as fungicide in agriculture field and we study about their microbial degradation.

MATERIALS AND METHODS

Collection of soil sample and physico-chemical analysis

The soil samples were collected from two different sites of Indira Gandhi Agriculture University Raipur of Chhattisgarh State. The samples were collected from agricultural fields growing mainly rice and vegetables. Soil samples were collected from agricultural lands having history of repeated hexaconazole and carbendazim applications. The soil samples were collected by using auger up to a depth of 15 cm. The collected air-dried soil samples were crushed and sieved through a 2 mm mesh to remove grasses, pebbles and stones, prior to analysis. Stored in the sealed plastic bags at room temperature. These stored samples were used for further experimentation.

The general physico-chemical properties of the soils were determined using standard analytical procedures.

Fungicides, chemical, Reagent

Based on the use-pattern of the fungicide in the Raipur district of Chhattisgarh State, two fungicides were selected in the present study. One fungicide was a technical grade of hexaconazole (95% purity), and the other was technical grade of carbendazim (97% purity). Both of these fungicides were purchased from the local fungicides suppliers in Bharat Krishi Kendra Raipur district of Chhattisgarh State. The analytical grade chemicals and reagents used in this work include Hydrogen peroxide, Sodium hydroxide, Potassium dichromate (1N), Diphenylamine indicator (0.02%), Phenolphthalein indicator, Ortho-phosphoric acid (85%), Ammonium thiocyanate, Sulphuric acid, Agar etc. from Hi-

media, and Glacial acetic acid, Hydrochloric acid, Sodium chloride, Ferrous ammonium sulphate (0.5N) etc. from Loba cheme pvt Ltd Mumbai(India).

Isolation and screening of fungicide resistant bacteria

The enrichment culture technique was used for the isolation of bacterial strains capable of utilizing hexaconazole and/or carbendazim as a sole source of carbon and energy. Fungicide mixed media were prepared by thoroughly mixing different volumes of technical grade fungicide with 100 ml media (MSM broth) as a sole carbon source, when the media were about to solidify (45-50°C). Different concentrations ranging from 5 to 200 mg/L of hexaconazole and carbendazim were added and checked for the growth of bacteria. One gram of each soil sample was suspended in 9 ml of distilled water and kept at room temperature for 24 hours. On the next day, 500 µl of the supernatant was spread on fungicide containing N-agar, LB and MSM petridishes, for the isolation of fungicide resistant bacteria. Solid N-agar, LB and MSM media were prepared, in which fungicide instead of glucose was added at a final concentration of 100mg/L. The samples were spread on N-agar, LB and MSM media containing different concentrations of HCZ (hexaconazole) and CBZ(carbendazim) separately and the petridishes were incubated at room temperature and 37°C. The petridishes were observed on the next day to till fourth day for the appearance of resistant colonies. The MSM medium plates containing separately HCZ and CBZconcentration ranging from 5 to 200 mg/L were streaked with fungicide resistant colonies and incubated at 37°C for 7 days. The concentration at which the isolate failed to grow even after 7 days of incubation was considered as minimum inhibitory concentration (MIC) for the given bacterial isolates.

Purification of potential bacterial isolates

A single isolated colony of the pesticide degrading bacteria was picked up with the help of sterilized wire loop and was streaked on LB agar medium. Each isolated strain was streaked at least 3 to 4 times on LB agar plates for purification. After the purified isolates were obtained, they were re-streaked on MSM agar medium containing fungicide (hexaconazole and carbendazim) for confirmation of isolates. The single colony of bacterial strain was inoculated in 100mL LB broth, incubated at 37°C then used for further characterization of isolates. The isolated and purified bacterial strains were stored under refrigeration after preparing slants or broth.

Maximum Tolerance capacity

The maximum tolerance is determining the capacity of the bacterial strain to degrade fungicide (hexaconazole & carbendazim) which is present along the media. The concentration of the compound hexaconazole and carbendazim was increased and then was incubated at 37°C for 7 days. The growth was monitored, at range up to which the bacterial strain was seen is considered as the maximum tolerance concentration of the compound. It's the maximum range up to which the bacteria can degrade the compound.

Molecular characterization and Phylogenetic analysis

The selected isolates was sent gene sequencing for identification of isolates on the basis of 16S rRNA gene sequence analysis. The isolated gene was amplified using 16sDNA universal primers16s forwardPrimer 16s Reverse Primer. The sequences of forward primer and reverse primer were 5'-AGHGTBTGHTCMTGNCTCAS-3'and 5'-TRCGGYTMCCTTGTWHCGACTH - 3', respectively. A phylogenetic tree was constructed using the partial 16S

rRNA gene sequences of the isolates and other closely related sequences (with the reference strains) obtained from chromous database.

RESULT

Physico-chemical analysis of soil samples

In the present study, two soil samples were collected from Indira Gandhi Agriculture University Raipur of Chhattisgarh State. Soil samples given as code no. S1 & S2 from Agriculture University Raipur and their physicochemical properties were determined. The basic physicochemical properties of the soil samples (Table 1) indicated the following characteristics the pH was found to be slightly acidic with the values ranging between 5.97–6.82, organic matter ranged between 0.30–0.40%, while moisture content ranged between 35–37%.

Table 1: Physico-chemical properties of soil samples.

Parameter	S1	S2
pH	5.97 ± 0.02	6.82 ± 0.14
Organic Matter (%)	0.40 ± 0.41	0.30 ± 0.22
Moisture Content (%)	35 ± 0.13	37 ± 0.02
Water holding capacity (%)	38.5 ± 0.5	29.2 ± 0.8
Texture (Sand: Silt: Clay)	75.7: 5.3:19	86.5: 4.5:9

The experiments were performed in triplicates and individual values are presented as Means ± standard deviation.

Isolation, characterization and identification of bacterial strain

Morphologically different bacterial strains were isolated from soil samples of fungicide contaminated sites of agriculture field, Indira Gandhi Agriculture University, India by enrichment method. The cultures were enriched by cultivating on MSM using hexaconazole/carbendazim as the sole source of carbon and energy. For long term maintenance, the bacterial strains were stored at -20 °C in 15% (w/v) glycerol. Out of these, 2 bacterial strains HCZ 1 & MBC1 (HCZ1-hexaconazole & MBC1-carbendazim) isolated from fungicide contaminated sites, were further characterized through polyphasic approach. The morphological and biochemical properties of the selected isolates are presented in table no.2 and 3. Morphologically, both isolates were spore-forming, non-motile, gram-negative rod. Based on biochemical tests, all the strains showed positive results for oxidase, catalase, nitrate reduction, Indole, citrate but negative for Voges-Proskauer test. Based on morphological, biochemical and partial 16S rRNA sequence (of isolate HCZ1 Only) analysis, the strain HCZ1 was identified as *Pseudomonas* species.

Table 2: Morphological characteristics of bacterial isolates.

Characteristics	Morphological characteristics	
	HCZ1 Strain	MBC1 Strain
Form/configuration	Irregular	Irregular
Elevation	Raised	Umbonate
Margin	Erose	Undulate
Pigmentation	Cream	Cream
Surface	Rough	Rough
Opacity	Opaque	Opaque
Gram's reaction	Negative	Negative
Cell shape	Rods	Rods
Size (µm)	1.0-1.2	1.0-1.2
Arrangement	Chains	Chains

Table 3: Biochemical characteristics of bacterial isolates.

Characteristics	Biochemical characteristics	
	HCZ1 Strai	MBC1 Strain
Oxidase	+	+
Catalase	+	+
MR	+	-
VP	-	-
Indole	+	+
H ₂ S Production	-	+
Amylase	+	+
Endospore staining	+	+
Nitrate reductase	+	+
Citrate utilization	+	+
Motility	-	-
Gelatine	+	+
Triple sugar iron agar	+	+

Maximum tolerance capacity

The isolates HCZ1 was show growth on maximum concentration (1200µl/L) of hexaconazole and the isolate CBZ1 was growth on maximum concentration (0.9 gm/L) of carbendazim. That means this concentration their maximum tolerance capacity of isolates.

Table 4: Different Concentration of hexaconazole & carbendazim, resistance to isolates strain.

Concentration of Hexaconazole (µl/L)	Growth of colony	Concentration of Carbendazim (g.m./L)	Growth of colony
100	+	0.01	+
200	+	0.05	+
300	+	0.1	+
400	+	0.2	+
500	+	0.3	+
600	+	0.4	+
700	+	0.5	+
800	+	0.6	+
900	+	0.7	+
1000	+	0.8	+
1200	+	0.9	+
1300	-	01	-
1400	-	1.5	-

Colony growth shown +, Colony not growth shown-

Molecular characterization of the isolates**Isolation gel electrophoresis and PCR of the Genomic DNA**

Genomic DNA was isolated from the sample, 16s-rDNA fragment was amplified using high-fidelity PCR polymerase. The PCR product was sequenced bi-directionally. The sequence data was aligned and analysed to identify the bacterium and its closest neighbors.

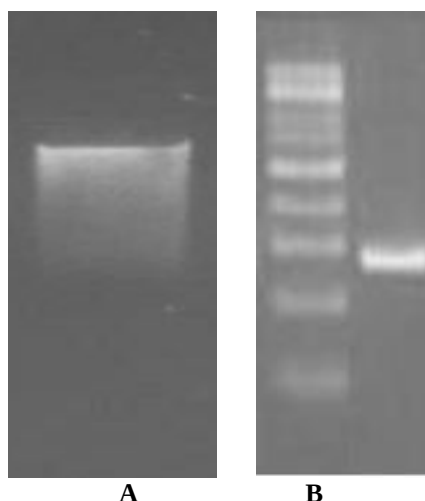


Fig. 1: (A) Genomic DNA from Bacterial sample loaded in 1% agarose gel. (B) PCR amplification of 16s rDNA fragment from Bacterial sample. The size of PCR amplified product is ~1.3kb

For genomic sequencing, sequencing mixture are prepared on the basis of standard protocol. In 10 μ l Sequencing reaction Big Dye Terminator ready reaction Mix (4 μ l), Template (100ng/ μ l) 1 μ l, Primer (10pmol/ λ) 2 μ l, Milli Q Water 3 μ l are involved.

Principle of PCR (25 cycles), Initially denaturation (96 $^{\circ}$ C for 5 min) of genomic DNA, and then hybridization (50 $^{\circ}$ C for 30 sec) of genomic DNA with primer their sequence was known. Elongation (60 $^{\circ}$ C for 1.30 min) it is last step of PCR. On the basis of 16S rRNA gene sequencing, sequences data of the isolated strain HCZ1 have a 1262bp and their detail sequence was given below.

Phylogenetic Analysis

On the basis of 16S rRNA gene sequences the isolated strain HCZ1 was identified as *Pseudomonas* species. Phylogenetic tree was constructed for HCZ1 strain with their sequences and their representative sequences obtained from Chromos database. Organisms *Pseudomonas* sp. Strain 4C3 (their accession no. MH000216.1), *Pseudomonas* sp. Strain 6H3 (MH000214.1), *Pseudomonas* sp. Strain 5B8 (MH000213.1), *Pseudomonas paralactis* strain MBP18 (MG952589.1), *Pseudomonas gessardii* strain 17C4 (MG269609.1), *Pseudomonas azotoformans* strain 5827 (KX418497.1), *Pseudomonas azotoformans* strain 58116 (KX418495.1), *Pseudomonas azotoformans* strain 58111 (KX418493.1), *Pseudomonas azotoformans* strain 58104 (KX418491.1), *Pseudomonas* sp. Strain KBL19 (MG576172.1) they were similar to *Pseudomonas* sp. strain 6H3 16S ribosomal RNA gene, partial sequence Sequence ID: MH000214.1. Phylogenetic tree was given in fig.2

Sl. No.	Organism Name	Accession No.	Percentage Match
1	<i>Pseudomonas</i> sp. Strain 4C3	MH000216.1	100%
2	<i>Pseudomonas</i> sp. Strain 6H3	MH000214.1	100%
3	<i>Pseudomonas</i> sp. Strain 5B8	MH000213.1	100%
4	<i>Pseudomonas paralactis</i> strain MBP18	MG952589.1	100%
5	<i>Pseudomonas gessardii</i> strain 17C4	MG269609.1	100%
6	<i>Pseudomonas azotoformans</i> strain 5827	9KX418497.1	100%
7	<i>Pseudomonas azotoformans</i> strain 58116	KX418495.1	100%
8	<i>Pseudomonas azotoformans</i> strain 58111	KX418493.1	100%
9	<i>Pseudomonas azotoformans</i> strain 58104	KX418491.1	100%
10	<i>Pseudomonas</i> sp. Strain KBL19	MG576172.1	100%

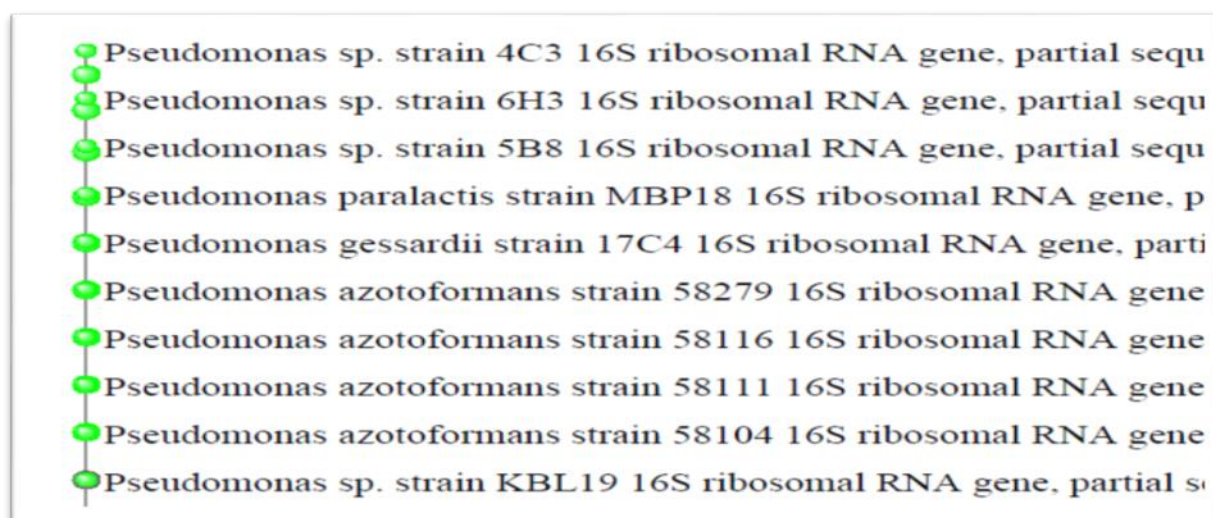


Fig. 2: Phylogenetic tree of *Pseudomonas* spp.

DISCUSSION

To increase the agricultural yield fungicide plays an important role, but only less than 1% of the pesticides are enough to kill the pests, remaining pesticides enter into the ground and surface water and causes environmental pollution and affect human health also.^[9,10] Thus some of the persistent pesticides were banned and some are modified without damaging to the environment. However, organophosphates are creating a lot of human health problems. Different kinds of bacteria present in soil are capable of degrading many persistent pesticides.^[11-14]

In the present study, the soil samples collected from two different sites of Indira Gandhi Agriculture University Raipur of Chhattisgarh State. The samples were collected from agricultural fields growing mainly rice and vegetables.

In present study enrichment conditions favoured the selective isolation of fungicide degrading bacteria from soil with a great degree of resistance towards different hydrocarbons. Two bacterial strains were isolated and screened, one potent strain (HCZ1) showing ability to degrade high concentrations of fungicide (100 µl/L) and second strain (CBZ1) showing ability to degrade high concentrations of fungicide (90 mg/L) were selected during present study through enrichment technique. Morphological characteristics of the isolates were studied (Table 5.1.2A). The degradation of hexaconazole (µl/L) observed respectively, from the initial control (0.05 µl/L). The isolate HCZ1 which showed maximum hexaconazole degradation was further sent for 16s rRNA identification. Genomic DNA was isolated from the sample. Genomic DNA from Bacterial sample loaded in 1% agarose gel. The ~1.3 kb/1.5kb, 16s-rDNA fragment was amplified using high-fidelity PCR polymerase. The PCR product was sequenced bi-directionally. The sequence data was aligned and analyzed to identify the bacterium and its closest neighbors. The selected isolate was gram negative the biochemical characteristics showed in table 5.2(B). The 16s rDNA reports revealed that the accession number was MH000216.1 closest homologue was found to be *Pseudomonas* sp. strain 6H3 16S ribosomal RNA gene (MH000214.1)

Based on morphological, biochemical and partial 16S rDNA sequence analysis, the strains HCZ1 was identified as *Pseudomonas* sp. strain 4C3 (MH000216.1). *Pseudomonas* sp. is one of the most studied bacteria as it can degrade a wide range of recalcitrant xenobiotic (Mulet et al., 2011) including fungicide.^[15-18]

The discoveries of microorganisms capable of tolerating or growing in high concentrations of Pesticides provide a potentially interesting avenue for treating hazardous wastes. Some investigations resulting in the identification of microbial isolates which are apparently responsible for the accelerated degradation of individual pesticides is necessary of which the 16s rDNA analysis is considered to be the most valid and widely used technique in the identification of microorganisms.^[14]

The present study revealed that the Hexaconazole was decreased significantly by *Pseudomonas* spp. However different degradation rates were observed. All the organisms could utilize Hexaconazole as a sole carbon source and had the bioremediation ability. Further it was observed that the soil isolate exhibited a *Pseudomonas* sp. good growth rate and higher biodegradation efficiency towards Hexaconazole, and compared to the other isolate it is suggested that the detoxification metabolism occurs when a microorganism uses the pesticide as carbon source. Earlier studies have reported that the isolated *Pseudomonas paralactis* strain MBP18 strain possesses a strong ability for hexaconazole degradation. This strain contains a plasmid which was believed to be responsible for the degradation of hexaconazole carrying the degrading gene. Such plasmid could be transferred to another bacterial strain in the environment and provide it with additional pesticide degradative ability and potentially selective advantage under a given environmental state.

CONCLUSION

The present study revealed that the *Pseudomonas* spp. is ubiquitously present in soil and can be exploited for Hexaconazole degradation. This study is beneficial as by having a detailed knowledge about the pesticide degrading bacteria because of the vast accumulation of xenobiotic on the earth and its non-consumption directly by the plants and other organism. Such identification and characterization of degrading bacterial species is one of the very good strategies for environmental clean-up. Microbial biodegradation is a major process leading to transformation of organic chemicals in soil, and hence bioremediation is viewed as the most attractive approach to the ultimate destruction of exogenous agricultural chemicals added to cultivated soils. Recombinant DNA technology is a relatively new and fast growing area where the bacterial plasmids are being relatively used as cloning vehicles, because of its small size and relaxed mode of replication, resulting in multiple copies in a cell. Such plasmids increase the biochemical versatility of the host bacterium, extending the range of complex organic compounds used as sole sources of carbon and energy. In bacteria, these determinants are often found on plasmids, which have facilitated their study at the molecular level.

REFERENCES

1. Debarati, P.; Gunjan, P.; Janmejy, P.; Rakesh, V.J.K., Accessing Microbial Diversity for Bioremediation and Environmental Restoration, Trends in Biotechnology, March 2005; 23(3): 135-142, ISSN 0167-9430.
2. Jain, R.K.; Kapur, M.; Labana, S.; Lal, B.; Sarma, P.M.; Bhattacharya, D.; Thakur, I.S. (2005). Microbial Diversity: Application of Microorganisms for the Biodegradation of Xenobiotic, Current Science, (July 2005); 89(1): 101-112, ISSN 0011-3891.
3. Finley, S.D.; Broadbelt, L.J. & Hatzimanikatis, V. (2010). In Silico Feasibility of Novel Biodegradation Pathways for 1,2,4-Trichlorobenzene, BMC Systems Biology, February 2010; 4(7): 4-14, ISSN 1752-0509 G.S.U.S. Geological Survey Circular 1150.
4. Mishra, V.; Lal, R.; Srinivasan, S., Enzymes and Operons Mediating Xenobiotic Degradation in Bacteria, Critical Reviews in Microbiology, 2001; 27(2): 133-166, ISSN 1040-841x.

5. Gavrilesco, M. (2005). Fate of Pesticides in the Environment and its Bioremediation, Engineer in Life Science, (December 2005); 5(6): 497-526, ISSN 1618-2863.
6. Rama, E.M.; Bortolan, S.; Vieira, M.L.; Gerardin, D.C.C.; Moreira, E.G. Reproductive and possible hormonal effects of carbendazim. *Regul. Toxicol. Pharm.*, 2014; 69: 476-486.
7. Silva, R.C.; Barros, K.A.; PAVAO, A.C. Carcinogenicity of carbendazim and its metabolites. *Quím. Nova [online]*, 2014; 37(8): 1329-1334.
8. Luong, T. T., Nguyen, T. H. T., Nguyen, T. D., Le, V. T., Pham, T. H. T., Ho, T. T., & Nguyen, N. L., Degradation of triazole fungicides by plant growth-promoting bacteria from contaminated agricultural soil. *Journal of Microbiology and Biotechnology*, 2023; 34(1): 56.
9. Bhatt, D., Srivastava, A., Srivastava, P. C., & Sharma, A., Evaluation of three novel soil bacterial strains for efficient biodegradation of persistent boscalid fungicide: Kinetics and identification of microbial biodegradation intermediates. *Environmental Pollution*, 2023; 316: 120484.
10. Mohanta, M. K., Saha, A. K., Zamman, M. T., Ekram, A. E., Khan, A. S., Mannan, S. B., & Fakruddin, M., Isolation and characterization of carbofuran degrading bacteria from cultivated soil. *Biochem Cell Arch*, 2012; 12(2): 313-320.
11. Rahman, M. A., Arefin, A. S., Saha, O., & Rahaman, M. M., Isolation and identification of pesticides degrading bacteria from farmland soil. *Bangladesh Journal of Microbiology*, 2018; 35(2): 90-94.
12. Mukhopadhyay, A., Barman, M., Majumder, S., & Singh, N. S., Biodegradation of the Fungicide by Bacteria in Soil. In *Land Remediation and Management: Bioengineering Strategies*, 2023; pp. 127-146). Singapore: Springer Nature Singapore.
13. Yadav, S., Verma, S. K., & Chaudhary, H. S., Isolation and characterization of organophosphate pesticides degrading bacteria from contaminated agricultural soil. *Online Journal of Biological Sciences*, 2015; 15(3): 113.
14. Rohilla, S. K., & Salar, R. K., Isolation and characterization of various fungal strains from agricultural soil contaminated with pesticides. *Research Journal of Recent Sciences ISSN*, 2012; 2277: 2502.
15. Satapute, P., & Kaliwal, B., Biodegradation of the fungicide propiconazole by *Pseudomonas aeruginosa* PS-4 strain isolated from a paddy soil. *Annals of microbiology*, 2016; 66(4): 1355-1365.
16. Latifi, A. M., Khodi, S., Mirzaei, M., Miresmaeili, M., & Babavalian, H., Isolation and characterization of five chlorpyrifos degrading bacteria. *African Journal of Biotechnology*, 2012; 11(13): 3140.
17. dos Santos, J. C., Batista, I. H., Barroso, H. S., Lima, J. M. S., Neto, P. D. Q. C., Ghelfi, A., & Pereira, J. O., Biodegradation of the fungicide carbendazim by bacteria from *Coriandrum sativum* L. rhizosphere. *Acta Scientiarum. Biological Sciences*, 2017; 39(1): 71-77.
18. Megadi, V. B., Tallur, P. N., Mulla, S. I., & Ninnekar, H. Z., Bacterial degradation of fungicide captan. *Journal of agricultural and food chemistry*, 2010; 58(24): 12863-12868.