International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 6, 2019, pp.-1624-1627.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

## Research Article

# GREEN BEAN COFFEE AS NUTRIENT SOURCE FOR INSECTICIDE DEGRADING BACTERIA

**YADAV P.D., KADAM S., PATHAN H.A.* AND WAVHAL S.D.**
Ismail Yusuf College, Mumbai, 400060, University of Mumbai, Mumbai, Maharashtra, India
*Corresponding Author: Email - hinaa_123@yahoo.com

Received: April 07, 2019; Revised: June 24, 2019; Accepted: June 26, 2019; Published: June 30, 2019

**Abstract-** The main criteria of this research were to isolate organism from green coffee bean which can degrade insecticide (chloropyrifos). Coffee bean crushed and inoculated in MSM medium incubated at static condition on shaker for 1 week then transfer to fresh MSM medium of one-week incubation each. Then the organism was isolated on MSM agar medium by T plate method & spread plate method. Then the organism was characterized by biochemical test. This organism was characterized by CSIR-NCL Pune for 16s RNA sequencing. The organism was found to resemble (*Enterobacter chuandaensis*) by 16s RNA. This organism was exposed to different concentration of chlorpyrifos(2%,1%,0.5%,0.3%,0.2%) and spectrophotometer analysis was carried out and best result of degradation was obtained in 0.5%. result showed that coffee bean was an adequate nutrient source for bacterial growth and it significantly enhanced chlorpyrifos biodegradation.

**Keywords**- *Coffee, Insecticide, Biodegradation*

**Citation:** Yadav P.D., *et al*., (2019) Green Bean Coffee as Nutrient Source for Insecticide Degrading Bacteria. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 6, pp.-1624-1627.



**Academic Editor / Reviewer:** Amit Fulzele

### Introduction

Insecticides are used for various purposes such as in agriculture, medicine, industry and by consumers. Insecticides are to be a major factor behind the increase in the agricultural productivity. Insecticides have the potential to significantly alter ecosystems and environment many are toxic to humans and/or animals. Chlorpyrifos belongs to organophosphorus category of pesticides. It is acutely toxic and their mode of action is by inhibiting acetylcholine esterase, an important enzyme in the nervous system. On exposure to these classes of pesticides, the enzyme is unable to work, thus causing accumulation of acetylcholine, which interferes with the transmission of the nerve impulse at the nerve ending. In humans it causes the following symptoms- general weakness, headache, salvation, nausea, vomiting, diarrhea, abdominal cramps and tumor. It also hampers the reproductive system of the humans Increase in the world population in turn has tremendously increased the need for the food which is the result of agricultural activities. Hence, farmers have no scope to lose their produce due to pest attack as a result they are forced to use pesticides. These pest control chemicals are xenobiotic and persistent in nature and remain in the environment for a very long time. This results in soil pollution and loss of soil fertility. Pesticides not only affect the point where it is being applied but may flow far away into the water bodies carried away by the agricultural runoff and sometimes percolate down into the ground water or enter into food, produced by the plant through root absorption or can also spread in the air after its spray. Chlorpyrifos can enter the body through mouth, lungs, and skin. chlorpyrifos quickly passes from the intestines to the bloodstream, where it is distributed to the rest of the body, by breathing chlorpyriphos spray bit directly absorb in the lungs and it passes to the blood stream quickly. It may also enter your body through the skin, but the effect is not great. the chances of being exposed to harmful levels of chlorpyrifos this way is not as great. Exposure to infants represents a greater health risk than compare to adults.

### Degradation

The available information on biochemical pathways of pesticide degradation in composting is extremely scanty. Such information must be generated to determine whether the pesticide residue has been effectively eliminated and not just converted from one toxic form to another, which may not be detectable by the analytical method employed. The metabolites and degradative pathways of some pesticides have not been fully elucidated. Lack of detection of the parent pesticide may be a result of analytical constraints or the formation of intermediates not detectable by the method used. The majority of herbicides tend to be aromatic and hydrophobic, leading to their partitioning into plant tissue. Thus, the use of composted yard waste previously treated with an herbicide or an insecticide formulation is potentially unsafe for use on edible crops. The susceptibility of pesticides to biological degradation is extremely variable because of differences in molecular structure as well as in chemical and physical properties. Aromatic compounds such as 2,4-D, MCPA, mecoprop, and dicamba are halogenated, and their recalcitrance in the environment varies. Green coffee bean is processed by wet or dry methods for removing the outer pulp and mucilage and have an intact wax layer on the outer surface. They are green when immature. When mature, they have a brown to yellow or reddish color and typically weigh 300 to 330 mg per dried coffee bean. Green coffee beans contain such as caffeine, deter many insects and animals from eating them. It also contributes both to the flavor of the coffee bean when it is roasted.

### Material and methods

1. Take 10g of green coffee bean, crush it with the help of mortar & pestle
2. Take crushed coffee bean into conical flask having 100 ml of MSM medium
3. Incubate at 30 °C & shaken at 100 rpm for 7 days
4. After 7 days transfer 2 ml to100ml fresh MSM and incubate at same condition.
5. Continue this step for 5 times.
6. Take loopfull from the fifth transfer & plate on MSM agar and incubate for 24 hrs at 30°C.
7. Observe the colony characteristics
8. 3 colony selected for biochemical test/ gram staining/ catalase etc.

9. For degradation standard dilution table were use.
10. Incubate all the tubes at 30 °C for 2 days.
11. Take absorbance at 530nm.
12. Repeat the steps 3 times and note down the absorbance

**Experimental Work**

**Biochemical & Physiological characterization**


Fig-1 Crushing of green coffee bean for isolation

7 day incubation 21 days incubation 35 days incubation

Fig-2 Crushed coffee bean into conical flask having 100 ml of MSM medium & Incubate at 30°C & shaken at 100 rpm for 7 days

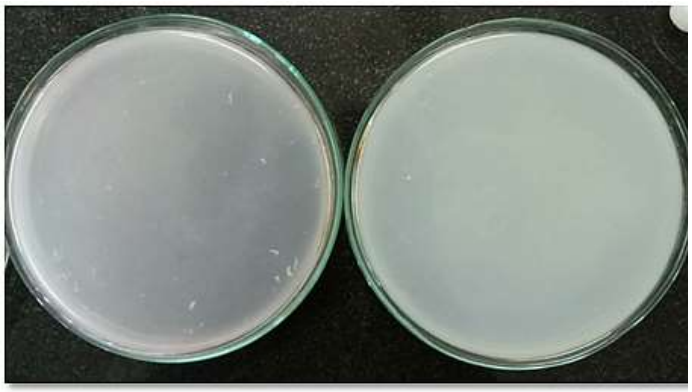

Fig-3 Cultured on MSM agar:

Spread plate technique T plate technique

Fig-4 Isolation can be done by 2 method

**Degradation of chlorpyrifos using isolated bacteria**

Standard dilution table were use ,3 colonies were selected for degradation.

Day 1: different concentration of chlorpyrifos were used for degradation:

Fig-5 Colony 1

Fig-6 Colony 2

Fig-7 Colony 3

Fig-8 After 2 days

Fig-9 After 4 day incubation

Fig-10 After 6 day incubation

## Observation

Table-1 Colony characteristics

| Characteristics | observation |
|---|---|
| Size | Pin point |
| Shape | Circular |
| Elevation | Flat |
| Margin | Complete |
| Opacity | Translucent |
| Color | white |
| Gram nature | |
| Morphology | |
| Arrangement | Single |

## Absorbance

Table-2 Absorbance of colony 1 at 530nm

| Sample | Colony 1 | | | | |
|---|---|---|---|---|---|
| Concentration | 2% | 1% | 0.5% | 0.3% | 0.2% |
| 0 Day | 1.694 | 0.857 | 0.843 | 0.834 | 0.794 |
| 2 Day | 1.425 | 0.765 | 0.785 | 0.813 | 0.785 |
| 4 Day | 1.365 | 0.623 | 0.654 | 0.754 | 0.721 |
| 6 Day | 1.245 | 0.452 | 0.521 | 0.692 | 0.698 |

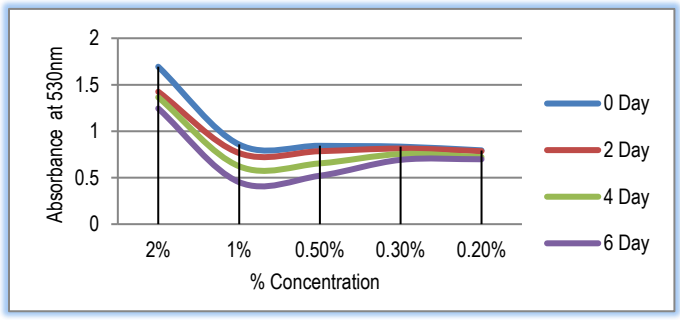


Fig-11 O.D of colony 1

Table-3 Absorbance of colony 2 at 530nm

| Sample | Colony 2 | | | | |
|---|---|---|---|---|---|
| | 2% | 1% | 0.5% | 0.3% | 0.2% |
| 0 Day | 1.564 | 0.857 | 0.824 | 0.854 | 0.754 |
| 2 Day | 1.495 | 0.796 | 0.795 | 0.793 | 0.727 |
| 4 Day | 1.465 | 0.652 | 0.601 | 0.753 | 0.695 |
| 6 Day | 1.355 | 0.573 | 0.589 | 0.687 | 0.675 |

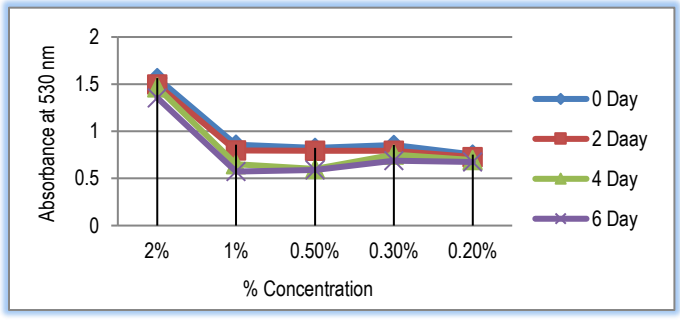


Fig-12 O.D of colony 1

Table-4 Absorbance of colony 3 at 530nm

| Sample | Colony 3 | | | | |
|---|---|---|---|---|---|
| | 2% | 1% | 0.5% | 0.3% | 0.2% |
| 0 Day | 1.465 | 0.796 | 0.754 | 0.754 | 0.785 |
| 2 Day | 1.439 | 0.721 | 0.732 | 0.732 | 0.658 |
| 4 Day | 1.356 | 0.652 | 0.632 | 0.632 | 0.596 |
| 6 Day | 1.296 | 0.598 | 0.546 | 0.546 | 0.564 |

In colony 3, day 6 was found to be better degrading bacteria than compared to other days at absorbance 530nm. The organism was characterized by CSIR- NCL Lab Pune and it is confirmed to be *Enterobacter chuandaensis*

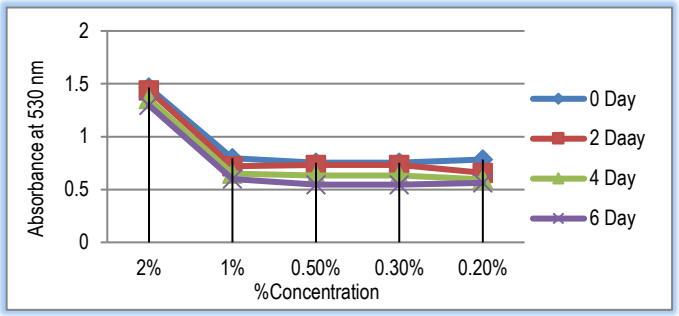


Fig-13 O.D of colony 1

## Conclusion

The main purpose of this research was to isolate bacteria from green coffee bean as nutrient source for insecticide degradation. The organism was grown in MSM medium after 5th transfer to fresh MSM of one-week incubation each. These microorganisms used green coffee bean as nutrient source. The organism is further check for biochemical nature and it was found that Indol, Methyl Red, Lactose, $H_2S$, Urease are Negative and Voges Proskauer, Citrate, Glucose, Maltose, mannitol, Catalase, Sucrose are Positive. This organism was exposed to different concentration of Chlorpyrifos (2%,1%,0.5%,0.3%,0.2%) and spectrophotometer analysis was carried out and the best result of degradation was obtained in 0.5%. Then the organism was send to NCL for characterization and organism was confirmed that *Enterobacter chuandaensis*. The sequence was analyzed by NCL and it was found to be:

>800RC_Seq 171

TTGATCCTGGGCTCAGATTGAACGCTGGGCGGCCAGGCCTAACACATGCAA
GTCGAAACGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAAC
GGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGC
CTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGG
CTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACT
GGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCC
TTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGATGAGGTTAATAACC
TCAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA
GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA
GCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCT
GGGAACTGCATTCGAAACTG

## Future Prospects

After degradation the compound of end product degradation was tried to be separated by TLC method to know the by product. The result obtained was not satisfactory so in future same method can be performed at higher level to know the end product and determine its future applications

## Application of research:

1.To carry out bioremediation
2. To use green coffee bean to degrade harmful insecticides
Defective green coffee bean is a readily available and relatively inexpensive material which could be used as nutrient source to enhanced organochlorine insecticide

**Research Category:** Insecticides

**Acknowledgement / Funding:** Authors are thankful to Ismail Yusuf College, Mumbai, 400060, University of Mumbai, Mumbai, Maharashtra, India

***Research Guide or Chairperson of research: Dr Heena Asif Pathan**
University: University of Mumbai, Mumbai
Research project name or number: MSc Thesis

**Author Contributions:** All authors equally contributed

**Author statement:** All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

**Study area / Sample Collection**: Grocery shop near Jogeswari, Mumbai

**Cultivar / Variety name:** Coffee

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

**References**


[1] Singh B.K. & Walker A. (2006) *FEMS Microbiology Reviews*, 30(3), 428-471.
[2] Don R.H. & Pemberton J.M. (1981) *Journal of Bacteriology*, 145(2), 681-686.
[3] Singh B.K. (2009) *Nature Reviews Microbiology*, 7(2), 156.
[4] Fogarty A.M. & Tuovinen O.H. (1991) *Microbiology and Molecular Biology Reviews*, 55(2), 225-233.
[5] Singh B.K., Walker A., Morgan J.A.W. & Wright D.J. (2003) *Appl. Environ. Microbiol.*, 69(9), 5198-5206.
[6] Aislabie J. & Lloyd-Jones G. (1995) *Soil Research*, 33(6), 925-942.
[7] Chaudhry G.R. & Ali A.N. (1988) *Appl. Environ. Microbiol*., 54(6), 1414-1419.
[8] Fogarty A.M. & Tuovinen O. H. (1991) *Microbiology and Molecular Biology Reviews,* 55(2), 225-233.
[9] Nawab A., Aleem A. & Malik A. (2003) *Bioresource technology*, 88(1), 41-46.
[10] Yang L., Zhao Y. H., Zhang B. X., Yang C. H. & Zhang X. (2005) *FEMS Microbiology Letters*, 251(1), 67-73.
[11] Yang C., Liu N., Guo X. & Qiao C. (2006) *FEMS Microbiology Letters*, 265(1), 118-125.
[12] Barragán-Huerta B. E. & Rodríguez-Vázquez R. (2010) Microbiology Book Series, (2), 1322-1327.
[13] Baby Sharma, Sonika Saxena, Aparna Datta and Sudipti Arora (2016) *Int. J. Curr. Microbiol. App. Sci*., (2016) 5(9), 742-749.
[14] Racke K.D. (1993) *Rev Environ Contam Toxicol.,* 131, 1-150.
[15] Whitney K.D., Seidler F.J. & Slotkin T.A. (1995) *Toxicology and Applied Pharmacology*, 134(1), 53-62.