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ISOLATION & CHARACTERIZATION OF CARBOFURAN PESTICIDE DEGRADING MICROORGANISMS FROM VARIOUS FIELD AREAS IN TAMIL NADU

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ABSTRACT: Samples were collected from various areas in Tamil Nadu and analyzed for its Carbofuran degradation. Soil samples were cultured on minimal media containing 1% Carbofuran pesticide concentration. The isolates were identified by using various techniques like Staining, Biochemical Analysis, Antibiotic Sensitivity and Heavy Metal Sensitivity Tests. Determination of phosphate activity and total proteins were done apart from effect of pH, temperature and concentration of pesticide on the bacteria. Thin layer chromatography was carried out to confirm degradation. Using HPLC technique, Carbofuran degradation was confirmed.

KEYWORDS: Carbofuran, Degradation, Proteins, Phosphates, Pesticides.

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1. INTRODUCTION

Agriculture is the backbone of Tamil Nadu. There is continuing increase in crop and horticultural farming in Tamil Nadu to meet the ever increasing market demand. This has led to modernization of agricultural practices which involves the use of both fertilizers and pesticides. Some of these chemicals pose threat to human health and have long term effect on the environment. Carbofuran is widely used for controlling the soil dwelling and leaf feeding insects, viz. corn root worm, wire worms, boll weevils, mosquitoes, alfalfa weevils and white grubs. Besides this it also exhibits

relatively high mammalian toxicity and, therefore, it has been classified as highly hazardous pesticide. In addition, several pesticides, including Carbofuran may fail to control pests after they are used continuously for a number of years resulting in economic loss from failed crops. This reduction in efficacy could result from the fact that the target pests may develop pesticide resistance analogous to the evolution of antibiotic resistant genes in microorganisms, and thus become insensitive to the pesticide. It has also become increasingly possible to isolate microorganisms that are capable of degrading Xenobiotics and recalcitrant compounds from environments polluted with toxic chemicals. Much of the recent research has focused on microbial ecology, biochemistry and physiology of microbes capable of biodegrading different organic and inorganic pollutants with little knowledge on genetic diversity of such bio degradative bacteria. This work focuses on the study of carbofuran degrading bacterial strains.

2. MATERIALS AND METHODS

1. **Sample collection**:

   Pesticide contaminated soils were collected from different areas in Tamil Nadu (Pazhani, Perampakkam, WCC College). Carbofuran 3CG was collected in local market in Thiruvallur. The carbofuran pesticide was carefully handled.

2. **Isolation of microorganisms from Soil Samples:**

   Nutrient media with Carbofuran pesticide (1g/100 ml) was used. Carbofuran was dissolved in methanol or acetone or acetonitrile and incubated at 37°C for 24-48 hrs. Minimal Media was used as degradation medium for bacteria. Cappuccino *et al*(2002).

3. **Identification of the Selected Bacterial Strains:**

   The selected strains were identified by Grams Staining Method. (Willey *et.al,* 2008)

4. **Biochemical Analysis:**

   The Isolated bacteria were subjected to the following Biochemical tests: Indole, Citrate , MR&VP, Oxidase, Catalase, Triple Sugar Iron Agar, Nitrogen Reduction, Dehydrogenase, Urease and Gelatinase Tests. (Murugesan *et al*, 2010) Cappuccino, J.G and Sherman,N (2002) ,Harold J. B (2002).

5. **Determination of Antibiotic &Heavy Metals Sensitivity Tests:**

   Antibiotic & Heavy Metal Sensitivity Tests of the Isolates was done using Muller Hinton Media. Different Antibiotic Discs like Azithromycin, Penicillin, Opcinen, Metamycin, Tetracycline & Vancomycin were used. Heavy metals like Copper, Manganese and Zinc were used. (Mujeeb et.al, 2012**)**

6. **Determination of Phosphatase Activity& Total Proteins:**

Phosphatase enzymes are involved in the hydrolytic breakdown of Carbamates as earlier mentioned (Omolo et al,2011). Their production by the isolated strains is an indication of the ability of the strains to hydrolyse amide bonds within the Carbamate structures for effective biodegradation. Determinations of the total proteins were done by Lowry's Method.

7. **Effect of pH, Temperature & Concentration of Pesticide:**

The effect of pH, Temperature & Concentration of pesticides for optimal growth of the bacterial isolates were determined. Different parameters like temperature (**10°C, 34°C, 40°C and 45°C**), pH (**2.0, 5.0, 9.0 and 11**) and the concentration of pesticides (**0.5, 1, 3 and 6**.) were determined. The absorbance was measured at 640nm in Colorimeter. Readings were taken periodically for 7 days (Omolo, et al 2011).

8. **Thin layer chromatography:**

To determine the Carbofuran degradation of bacteria by TLC method. The Solvents Chloroform: Ethyl acetate (3:1) Volume Ratio was used. The Retention factor (R_f) of the compound was calculated using the formula(Iyer.et, al (2009)

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

9. **High Performance Liquid Chromatography:**

A standard was prepared by dissolving the Pesticide in Acetonitrile to the final concentration. Carbofuran was detected at 254nm at a run time of 5 mins at a flow rate of 1ml per min using HPLC grade Acetonitrile (70%) and degassed water (30%) as the mobile phase. Sample of 2µl is injected at an oven temperature of 270°C

3. RESULTS AND DISCUSSION

1. Isolation of microorganisms from pesticide contaminated soil:

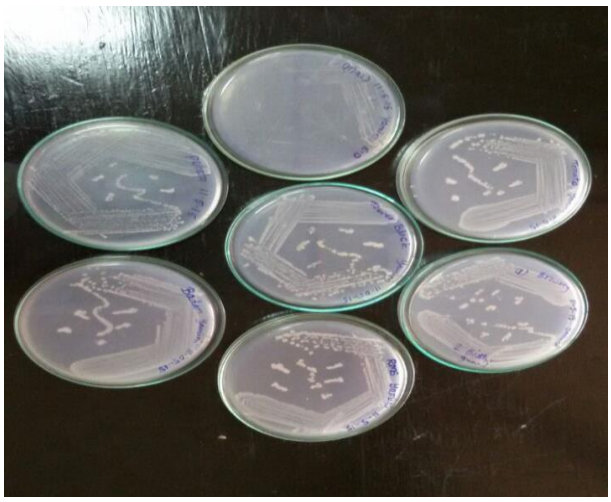

Fig: 1: Microorganisms isolated from the pesticide contaminated soil.

For isolation of organisms, Minimal Media was used which showed very high growth of organisms compared to Nutrient medium. The Minimal Medium was supplemented with Carbofuran and growth was observed within 24-48 hours. Six organisms were isolated which were able to degrade Carbofuran Pesticide

2. Gram's Staining:

The microorganisms isolated were identified using Gram staining method.

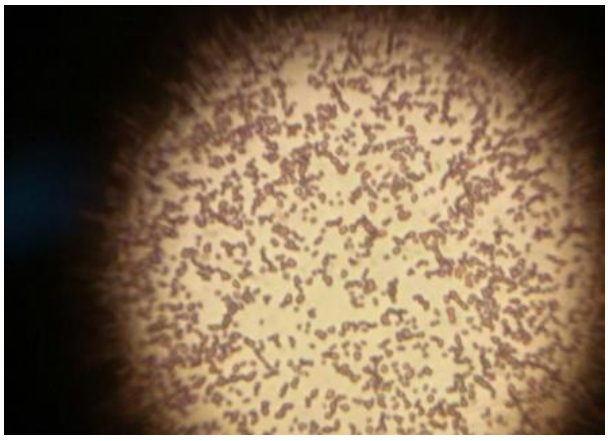

Fig-2: *Staphylococcus spp*.

3. Biochemical Analysis of the Isolates:

The following biochemical tests were done to identify the isolates found from the pesticide contaminated soil.

Table: 1Biochemical Tests

Biochemical tests	*Organisms*					
	Pseudomonas spp	***E. coli***	***Bacillus spp***	***Streptobacillus spp***	***Klebsiella spp***	***Staphylococcus spp***
Indole	+	+	+	+	+	+
Methyl Red	+	+	-	+	+	-
VogesProkauer Test	+	-	+	-	+	+
Citrate test	-	-	+	+	+	+
Oxidase test	+	-	-	-	-	-
Catalase test	+	+	+	+	+	+
Triple Sugar Iron Agar test	+	+	+	+	+	+
Sugar or carbohydrate test	+	+	+	+	+	+
Dehydrogenase Test	+	+	+	+	+	+
Nitrogen reduction test	+	+	+	+	+	+
Urease test	+	+	+	+	+	+
Gelatinase test	+	-	-	-	+	-

4. Antibiotic Sensitivity Tests:

The six bacterial isolates were spread on six LB agar plates separately. Then sensitivity discs of Ampicillin, Penicillin, Tetracycline, Metamycine, Opcinen and Vancomcycin were placed on the plates and were incubated overnight. The appearance of Zone of Inhibition around the discs showed the sensitivity of isolates to those antibiotics, while the growth of the isolates around the discs showed their resistance against those antibiotics. The zone of inhibition was measured for each disc.

Table -2 Antibiotic Sensitivity Tests

Organisms	Antibiotics					
	Azithromycin	**Penicillin**	**Vancomycin**	**Tetracycline**	**Metamycine**	**Opcinen**
Pseudomonas spp	0.5	-	-	-	-	-
E. coli	1.2	-	0.2	0.3	-	-
Bacillus spp	0.9	0.6	0.5	1.1	-	-
Streptobacillus spp	0.2		0.4	0.7	-	-
Kelbsiella spp	0.7	0.6	0.4	0.3	-	-
Staphylococcus spp	0.6	0.2	0.8	0.4	-	0.1

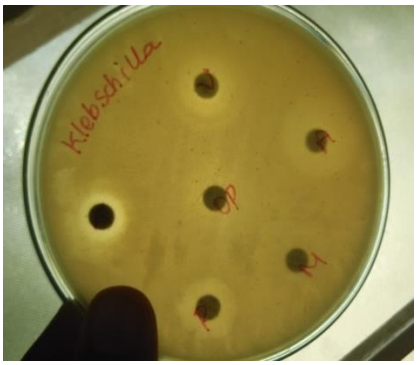

Fig -3: *Klebsiella spp*

5. Heavy Metal Sensitivity Tests:

All isolates were checked for their ability to resist heavy metals. For this purpose small filter paper disc (9mm diameter) was autoclaved and loaded with 1% solution of copper, manganese and zinc and allowed to dry for one hour. These discs were placed on the surface of the inoculated nutrient agar plates at 37 ºC for 24 hours Mohanta., *et al*(2012) .The clear zone of inhibition around the disc indicates that the organisms were inhibited by the heavy metal, whereas growth around the disc indicates that the given organism was resistant to the heavy metal. (Murugesan,.et al,2010).

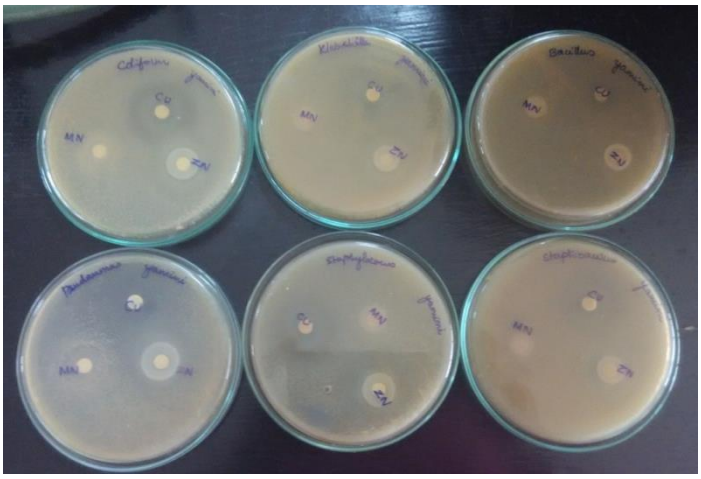

Fig-4: Heavy Metal Sensitivity

(*Streptobacillus spp, Staphylococcus spp, Bacillus spp, Pseudomonas spp, Klebsiella spp and E .coli.)*

Table -3 Heavy Metal Sensitivity Tests

Organisms	Metals		
	Copper	Manganese	Zinc
Pseudomonas spp	0.7	0.8	1.1
E. coli	1.2	0.7	0.6
Bacillus spp	0.6	0.2	0.4
Streptobacillus spp	0.9	-	0.8
Kelbsiella spp	1	0.3	0.8
Staphylococcus spp	0.9	0.2	0.4

6. Phosphatase Activity Tests:

The phosphatase activity was estimated using the amount of p -Nitro Phenolate produced which was determined against a p -nitro Phenolate standard curve. All the isolates had phosphatase activity which varied among individual isolates

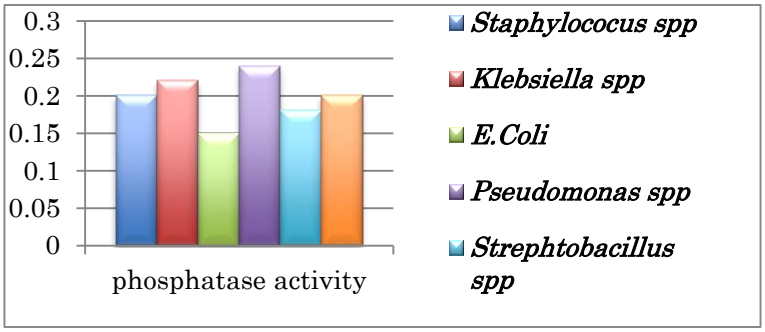

Fig-5: The concentration of p -nitrophenolate produced by isolates from 50mM p - nitro phenyl phosphate in 30 minutes.

Relationship between the total protein produced by different isolates and phosphatase activity determines the amount of p-nitrophenolate produced. **(Omolo, *et al*2011)**

7. Determination of total proteins:

The total protein was estimated against a standard curve prepared using Bovine Serum Albumin (BSA).The protein content also varied among the isolates.

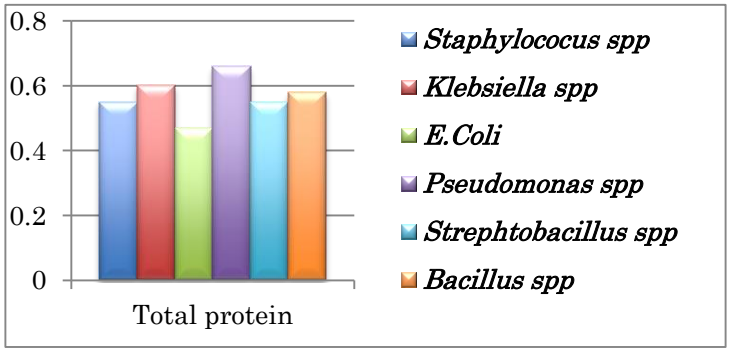

Fig: 6 The total proteins produced by different isolated cultures after 24 hours in a low phosphate media.

8. Effect of Temperature, pH and Concentration of Pesticide:

The effect of temperature, pH and concentration of the pesticide in the growth medium was tested. The optimum pH for the growth of the isolates was 7.0 and pH (2.0, 5.0, and11) restricted the bacterial growth. The highest growth rate (OD=0.38) was observed. The optimum temperature for the growth of the organism was found to be 34^{o}C and temperatures 10 ^{o}C, 40 ^{o}C and 45^{o}C restricted the bacterial growth. Maximum growth was observed at 34^{o}C (OD=0.53) after 10 hrs of culture and after 16 hours the OD started to decrease. The optimum concentration of pesticide for the growth of bacteria was found to be 1.

9. Thin layer Chromatography:

The Retention factor (R_f) of the compound was calculated using the formula

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

The TLC results showed that the retention time of Pseudomonas spp is 0.19 and standard carbofuran is 0.70. The analysis showed the presence of breakdown products.**(Slaoui *.et al*2007).**

10. High Pressure Liquid Chromatography:

Carbofuran was biodegraded at the retention time of 5.863 minutes, and for bacteria retention times is 5.395 (Figure7&8). Using independent enrichment step with carbofuran as the sole carbon and energy, fourteen carbofuran-degrading bacteria were isolated out of which six organisms were used. The ability of the isolates to utilize carbofuran as sole carbon source was assessed using HPLC over a period of 120 days with the reduction in the concentration of carbofuran was determined against carbofuran standard curve. The extent of degradation varied among the isolates. Growth of isolates was also monitored against change in concentration of carbofuran.

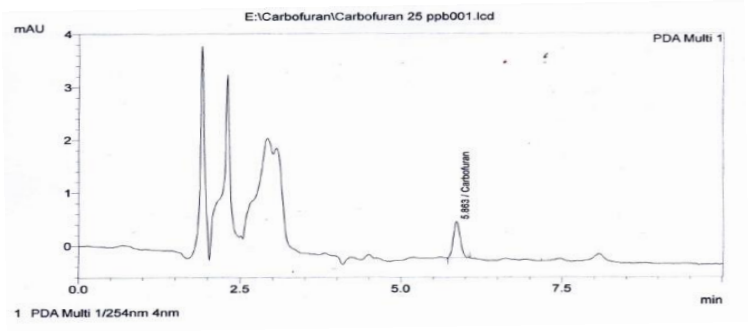

Fig: 7 HPLC STANDRAND FOR CARBOFURAN

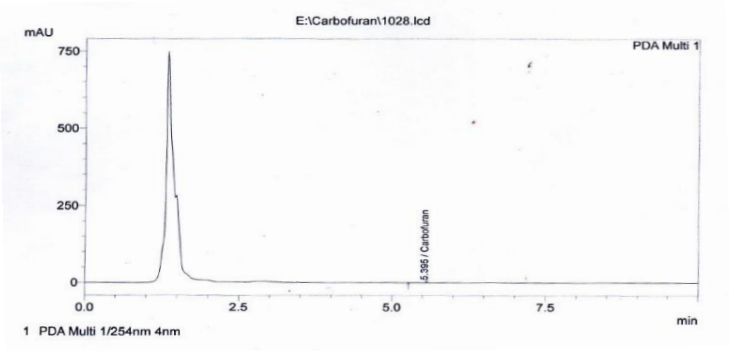

Fig: 8 HPLC FOR *Pseudomonas spp* DEGRADING CARBOFURAN

4. CONCLUSION

This paper suggests that the isolated organisms were able to flourish in the carbofuran pesticide used in farms by utilizing them as their source of energy when others source are limited or unavailable. From this study it has been confirmed that the isolated organisms were capable of degrading the pesticide. Several species of bacteria like Pseudomonas spp, Bacillus spp, Streptobacillus spp, Staphylococcus spp, Klebsiella spp and E.coli were isolated. These Bacteria increases the fertility of the soil as well as degrades the pesticide contamination.. Using HPLC, the ability of these isolated organisms to degrade the pesticide was determined.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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REFERENCES

1. Akhter.M.A.,Laz.T., (2013) Isolation and Molecular Characterization of Pesticide (Fenitrothion) Resistant Bacteria from Agricultural Field, IOSR Journal of Pharmacy, Volume 3, Issue 5
2. Bachman, J. and H. H. Patterson (1999). Photodecomposition of the Carbamate Pesticide Carbofuran: Kinetics and the Influence of Dissolved Organic Matter. EnviromentalScienceTechnology, 33:874-881
3. Cappuccino, J.G and Sherman,N (2002). Microbiology: a laboratory manual (6th ed) Pearson
4. Chapalmandugu, S. and Chaudhry, G.R. (1992).Microbial and biotechnological aspects of metabolism of carbamates and organophosphates.Critical Review Biotechnology, 12(5), 357–389.
5. Chaudhry, G.R. (2002). Induction of carbufuran oxidation to 4-hydroxycarbofuran by Pseudomonas sp.50432.FEMS Microbioogy. Letter, 214(2), 171–176.
6. Gibson, D. T and Subramanian, V. (1984).Microbial degradation of aromatic hydrocarbons. In: D.T.Gibson(ed), Microbial Degradation of Organic Compounds. Marcel Dekker, Inc.New York, NY, p.181-252.
7. Harold J. B (2002). Microbiological applications: a laboratory manual in general microbiology. 8th edition.1-478.

8. Hindumathy.CK and Gayathri.V., (2013) Effect of Pesticide (Chlorpyrifos) on Soil Microbial Flora and Pesticide Degradatin by Strains Isolated from Contaminated Soil, Bioremediation &Biodefradatin, Volume 4, Issue 2, 1-6
9. Janssen Dick B., Inez J. T. Dinkla, Gerrit J. Poelarends and Peter Terpstra (2005).Bacterial degradation of xenobiotic compounds: evolution and distribution of novel enzyme activities. Environmental Microbiology, 12:868–1882.
10. Kamal Zeinat M., Nashwa A.H., Fetyan A., Mohamed A. Ibrahim, and Sherif El- Nagdy (2008)Biodegradation and Detoxification of Malathion by of Bacillus Thuringiensis MOS-Australian Journal of Basic and Applied Sciences, 2(3): 724-732.
11. Khan,S.A., Hamayun. M., Bibi.N&Shewani.K.S.(2015). Isolation, screening and co-metabolism of polycyclic aromatic hydrocarbons by soil bacteria.American .eurasian J.agric .& Environ.sci.,vol-15(5)pp. 800-812.
12. Murugesan.A.G.,Jeyasanthi.T. andMaheswari.S,(2010) Isolation and Characterization of Cypermethrin Utilizing Bacteria from Brinjal Cultivated Soil, African Journal of Microbioligy Research, vol.(4)1, pp.010-013
13. Naphade. S.R., Annika.A.Durve, MeetaBhot, Jossy Varghese and Naresh Chandra., (2012) Isolation, Characterization and identification of Pesticide tolerating bacteria from Garden Soil, European Journal of Experimental Biology, 2(5):1943-1951.
14. Omolo.M.K., Magoma.G., Ngamau.K., and Muniru.T.,2012 Characterization of methomyl and carbofuran degrading bacteria from soil of horticultural farms in rift vally & central Kenya .African journal of envirmental science and technology vol- 6(2)pp. 104-114
15. Rakhee Nair, Priya.R.Iyer., (2009), Biodegradation-end of the Raod for Endosulfan, Environmental Science An Indian Journal, 4(2), (69-71)
16. Roger.P.A.,Simpson.I., Oficial.R., Ardales.S. andJimenez.R, (1994), Effects of Pesticides on soil and Water Microflora and Mesofauna in Wetland Ricefields: A summary of Current Knowledger and Extrapolation to temperate Environments, Australian Journal of experimental Agriculture, 34, 1057-68.
17. Shahgholi.H, (2014), Factors Controlling Defradation of Pesticides in the Soil Environment Review, TI Journals Agriculture Science Developments, Vol(3), No(8), pp.273-278

18. Sharif.D.I and Mithum. M, (2013), Selective Isolation of a Gram Negative Carbamate Pesticide Degrading Bacterium from Brinjal Cultivated Soil, American Journal of Agricultural and Biological Sciences 8(4): 29-256
19. Sriranjini.A.S., Michelle Samantha Fernandis, Savita Desai, (2012) Study and Identificatin of Potent Defrader of Chlorpyrifos Pesticide, International Journal of Research and Reviews in Pharmacy and Applied Science, 2(5) 847-858
20. SurekhaRani.M.,VijayaLakshmi.K., SuvarnalathaDevi.P., Jaya Madhuri.R., Aruna.S.,Jyothi.K., Narasimha.G. and Venkateswarlu.K, (2008), Isolation and Chracterization of a Chlorpyrifos-Degrading Bacterium from Agricultural soil and its growth response, African Journal of Microbiology Reseacrh, Vol (2) pp.026-031
21. Willey, J. M., L. M. Sherwood, and C. J. Woolverton. 2008. Prescott's microbiology, 8th ed. McGraw-Hill Companies, Inc., New York, USA.
22. Andrews,W.H.,G.A. June.P.S.Sherrod ,T.S.Hammack, and R.M.Amaguana. FDA bacteriological analytical manual, 8thed. AOAC international, Gaithersburg, MD.
23. Mujeeb.K.A., Riaz.A.,Mushtaq.S., Abbasi.H.M., and Ali.S. S.(2012). carbofuran degrading bacteria from different areas of Punjab as potential bioremediator of agricultural soil. sci.int.(Lahore).Vol - 24(3) pp-273-280.
24. Donovan .S., Taggart.M., Richards.N.,(2012).An overview of the chemistry, manufacture, environmental fate and detection of carbofuran.john wiley& sons ,inc.1sted pp 1-18.
25. Mohanta.M.K., A.K.Saha., Zamman.M.T., Ekram.A.E.,Khan.A.S., Mannan.S.B., M. Fakruddin.(2012). Isolation and characterization of carbofuran degrading bacteria from cultivated soil. Biochem.cell.arch.vol.12(2).pp 313-320.