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Fungi for Pesticides biodegradation

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Abstract

Fungi commonly known for diseases generating and fermentation agent, apart from this some fungi shows biodegradation agents. Pesticides are used for controlling plant diseases but when spraying the pesticides only 40% goes at target and rest of in soil environment. So the soil get contaminated and result of this useful microflora are affected and soil fertility decline. So some fungi tested against the pesticides viz. Cypermethrin-25 EC (insecticide), Carbendazim (Fungicide) and 2-4D (Herbicide) Resulting fungi *Trichoderma viride, Trichoderma koningii, Penicillium chrysogenum* and *Rhizopus stolonifer* are showing capacity to elimination of pesticides from soil. The HPLC, GC-ECD and Chemical oxygen demand (COD) result showed *Trichoderma viride* and *Trichoderma koningii* showed notable removal of pesticides in minimal mineral salt medium and *Penicillium chrysogenum* and *Rhizopus stolonifer* showed moderate removal of pesticides.

In modern days high yielding varieties leads to intensive agriculture. Thus use of agricultural chemicals becomes imminent in present day in agriculture. The intensive use of pesticides causes concern both to public and scientists all over the world. Most of the pesticides are organic or inorganic chemicals which have longer persistence in the environment. Despite the beneficial impacts of pesticides in improving and stabilizing agricultural productivity by control of obnoxious weeds, fungal diseases and insects, these all organic chemicals are known to contaminate soil ecosystem and pose threat to balance equilibrium among various groups of microorganisms in soil, which play an important role in recycling plant nutrients. Such important process like mineralization, nitrification and phosphorus recycling are dependent much on various groups of organisms in the soil. Enzymes mediate many processes occurring in soil. Soil enzymes contain free enzymes, immobilized cellular enzymes and other enzymes within microbial cells. These enzymes are derived from microorganisms, plant roots and soil animals which play an important role to the degradation of organic matter and xenobiotics such as pesticides. Soil enzymes and microorganisms both are useful for describing and understanding the ecosystem quality and the interactions among

subsystem and to assess the effect of various inputs on soil health. During a cropping season more than one type of pesticide reaches to soil and act on non-target microorganisms. Hence, examination of such interaction effect of the various pesticides or individual effect of these pesticides is warranted for the biochemical transformations which are of paramount importance in maintaining the soil fertility. Researches carried out the effect of pesticides on soil enzymes determined by the factors like, chemical and recalcitrant nature of the pesticides and physical and chemical properties of soils. Variation among soil enzymes with regard to their response to different pesticides is also evident. Results of such studies have indicated both inhibitory and promontory effect of different pesticides.

Microorganisms are used for the degradation of both soluble and non-soluble contaminates in soil. Microorganisms including fungi such as *Trichoderma vigratum*, *Aspergillus sydowii* and *Penicillium* sp. which is ability to degrade organochlorine pesticide in soil[3,4]. Pesticides in soil and water can be biodegraded and is the primary mechanism of pesticide breakdown and detoxification in many soils[9].

The first *Penicillium* strain described as capable to degrade 2, 4-D was isolated from tropical soil at Brazil[10]. This strain was reported as to present a great potential for 2, 4-D bioremediation. *Penicillium* strain removes about 29.80% of 2, 4-D from an initial concentration of 100 mg/l of a complex medium (nutrient broth).

The principal reaction involved in the pesticides degradation leads to loss of insecticidal, fungicidal and herbicidal activity and conversion into secondary metabolites. The intermediate of these pesticides would convert into simpler environmental friendly compounds on long acclimation with potential microorganisms. Therefore bioremediation of pesticides by isolated above microorganisms could be used for remediation of pesticides contaminated soil and water.

Chemicals :

There were three different types of technical grade Pesticides selected for the Microbial degradation study, including Cypermethrin-25EC, Crbendazum (Bavistin) and 2, 4-D belonging to group of insecticides, fungicide and herbicide respectively.

Insecticides: Cypermethrin-25EC :

The Cypermethrin-25 EC (BASF India Lid.) was purchased locally from Mondha market, Agriculture Shop, Aurangabad.

Composition :

Cypermethrin-25 EC Technical: (70% basis) 36% w/w, Surfactants- Cresilax AE1, AE2, AE3, (Calcium salt of Alkyl benzene sulphonate, Alkyl phenol Ethoxylate, Tri glyceride Ethoxylate): 10.00% w/w., Solvent-C-IX :54.00 w/w, Total 100.00% w/w.

Chemical formula: $C_{22}H_{19}O_3NCl_2$

Cypermethrin IUPAC Name: [(RS)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-1,1-dimethyl-cyclopropanecarboxylate.]

Fungicide: Carbendazim (Bavistin 50% WP) :

The Bavistin (BASF India Lid.) was purchased locally from Mondha market, Agriculture Shop, Aurangabad.

Chemical formula: $C_9H_9N_3O_2$.

IUPAC name: Methyl benzimidazol-2-ylcarbamate.

Chemical Composition: W/W

1) Carbendazim a.i. = 50%.
2) Sodium salt of sulphonic acid condensation product =08%
3) Sodium salt and Alkyl naphthalene = 08%
4) China clay (Filter) = q.s.
   Total= 100%.

Herbicide: Electron 2, 4-D Ethyl ester 38% EC. (Deridayal Agrochemical)

Composition :

1) 2, 4-D Ethyl ester. = 40% W/W.
   (Based on 86% W/W as 2, 4-D acid a.i.)
2) Emulsifiers (cal. Salt of Alkyl aryl =05.00 Sulphonate and poly oxy ethylene ether) Solvent (Aromax) =50%.
3) Total=100%.

Chemical formula: $Cl_2C_6H_3OCH_2COOH$ or $C_8H_6Cl_2O_3$

IUPAC name: 2, 4-dichlorophenoxyacetic**.**

Collection of soil samples :

The soil samples were collected from the different sites of Cotton, Brinjal and wheat cultivated field from Belora Dist. Jalna (M.S) India. These fields were already sprayed with Pesticides mainly Cypermethrin-25 EC, Carbendazim and 2, 4-D for past few years. The soil samples were collected in sterile polythene bags for further study.

Soil enrichment Technique for isolation of Pesticides degrading fungi :

Soil samples collected from the top 0-15 cm of field plots and were air dried and maintain 20% (w/w) moisture content. Fifty grams of each sample was placed in six glass plates and covered to maintain moisture conditions. The soil samples were treated with mix aqueous solution of Cypermethrin-25 EC, Carbendazim and 2, 4-D to get final concentration 1000 ppm by mixing gently and incubated at room temperature for two weeks. The moisture content was maintained using distilled water. The pesticides treatment was repeated three times for every two week of time interval.

Isolation of pesticide degrading microorganisms :

The enriched culture method was used to isolate the pesticides degrading microorganisms from soil. Enrichment of pesticides degraders were carried out in 150 ml minimal minerals salt medium in 250 ml conical flask. The medium was sterilized at 121°C for 30 min. Followed by addition of 1% (w/v) of pesticides separately as a sole source of carbon and energy. Consequently, second enrichment was carried out by transferring 1 ml of from first enriched flask culture into freshly prepared and sterilized 50 ml of minimal minerals salt medium and was incubated for same growth conditions. Isolation was done on sterile minimal minerals salt agar media plates with 0.25% pesticides. After 3 days incubation different types of colony were

observed and pick up isolated colonies and maintain on slants for further study.

After two successive enrichments isolation was carried out on sterile minimal mineral salt medium plates. Six fungi were found to be highest growth rate and they isolated and maintain on sterile slant of potato dextrose agar.

The different types of media were used for the isolation, maintenance of microorganisms and microbial degradation study. For isolation and degradation study Minimal minerals salt medium was used and for maintenance fungi Potato dextrose agar was used.

Minimal minerals salt nutrient culture medium :

A synthetic medium was used containing $(NH_4)2SO_4$-2 g, KH_2PO_4-3g, $MgSO_4.7H_2O$- 0.5g, glucose- 3g, microelements minerals solution -2 ml (Cooney and Levine, 1972) and distilled water 1 litre. The P^H was adjusted at 7 and media was sterilized at 121ºC for 15 minutes.

The fungi were identified in Department of Botany, Research laboratory, Government institute of science, Aurangabad (MS) India, using manual of soil fungi - Joseph C. Gilman.

Pesticides spiking :

Erlenmeyer flasks (250 ml) and nutrient culture media were autoclaved for 20 minutes at 121ºC. A 500µl acetone containing pesticide at varying concentrations separately was aseptically added to autoclaved dried Erlenmeyer flasks allowing the acetone to evaporate. After complete evaporation of acetone from the Erlenmeyer flasks, 100ml culture media was added under laminar flow hood so as to reach the desired pesticide concentration[1].

Scale-up technique :

1ml of 24 hours old four bacterial sub cultured suspension inoculated in separate 250ml Erlenmeyer flasks containing bacterial nutrient culture media with 50 mg/L concentration of cypermethrin and control was served without bacteria suspension and 1ml four days old four fungus mycelia/spore suspension was inoculated into separate 250ml Erlenmeyer flasks containing fungal nutrient culture media with 50 mg/L concentration of cypermethrin and control was served without fungus spore suspension. The microbial culture inoculated flasks were kept in orbital shaker at 30ºC with 160 rpm (rotation per minute) for 14 days. After 14 days of incubation period, 1 ml of this 14 days old cypermethrin degraded culture media and 100 mg/L of cypermethrin concentration was added into another 250 ml of Erlenmeyer flasks containing nutrient culture medium and control was served without microorganism. The flasks were again kept on orbital shaker at 30ºC with 160 rpm for another 14 days incubation period. Likewise, the microbial culture was sub cultured into other nutrient culture media containing Erlenmeyer flasks with cypermethrtin concentration of 150 mg/L and was kept on orbital shaker at 30 ºC with 160 rpm for increasing a total incubation period of 42 days. At this stage, the isolated microorganisms were found adapted to Cypermethrin-25 EC and by assessing the

cypermethrin as a sole source of carbon for growth and maintenance.

The same technique and same microorganisms were used for Carbendazim and 2,4-D degradation. The concentrations taken for Carbendazim and 2,4-D degradation study were Carbendazim was 100mg/l, 200mg/l and 300mg/l and 300 mg/l, 600 mg/l and 900 mg/l respectively with help of above mentions scale up process technique in controlled laboratory condition.

Chemical oxygen demand (COD) :

COD was determined as the oxygen required for chemical oxidation of organic matter with the help of strong chemical oxidant. (Reflex condensation with $k_2cr_2o_7$ methods using manual of water and waste water analysis, NEERI). COD was determining by refluxing sufficient diluted culture filtrate of microorganisms for 2 hours, in the presence of $HgSo_4$ and Ag_2So_4 in concentrated H_2So_4. After refluxing residual $K_2Cr_2O_7$ was estimated by titrating content with .01N ferrous ammonium sulphate using a ferroin as indicator with wine red color which was the end point.

Chemical oxygen demand is method to determine the overall chemical organic load of the water. It is better measures water organic load as well as non-biodegradable organic chemicals. COD data is helpful in knowing toxic conditions and the presence of biological resistant organic material in water. COD analysis of samples was done as per the standard procedure laid down in Book of Chemical and Biological Methods for water pollution study[11].

Procedure :

1. In COD flask, 20 ml of effluent was taken.
2. A pinch of Ag2SO4 and Hgso4 was added.
3. To it 30 ml of Conc. Sulphuric acid was added. Contents were refluxed for 2 hours on hot plates.
4. After which, the flask were removed and cooled and distilled water was added to make the final volume up to 140 ml.
5. Two to three drop of ferroin indicator were added, mixed thoroughly and titrate with 0.1 N Ferrous Ammonium Sulphate (FAS). A Blank with distilled water using same quantity of the chemicals was used

Calculation :

$$\text{COD in mg/l} = \frac{(b\text{-}a) \times \text{N of FAS } (0.1) \times 1000}{\text{ml of sample.}}$$

Where:

b= Amount of FAS needed for blank.

a= Amount of FAS needed for Sample.

Residual quantification analysis of Cypermethrin by Gas Chromatography with electronic capture detector (GC-ECD):

After every 14 day's interval the final concentration of cypermithrin was determined by GC-ECD methods. The solution mixture was extracted with dichloromethane; the organic layer was obtained and it was dried and re dissolved in *n*-hexane. The GC conditions were as follows: electron capture detector with SPB-5 capillary column, injector/ interface temperature 260°C, oven temperature 240°C, detector temperature-300°C, and N2 carrier gas 1 ml/min.

Detection of cypermethrin metabolites by Gas chromatography and Mass spectroscopy (GC-MS):

Culture filtrate of medium containing cypermethrin was extracted with dichloromethane. The dichloromethane extract was evaporated and the residue was dissolved in acetone. The extracts were analyzed by GC-MS (Doctors Analytical laboratory Pvt. Ltd. Pune). The GC–MS analysis was performed in electron ionization (EI) mode (70 eV) with an Agilent gas chromatograph equipped with an MS detector. A HP-1701 capillary column (30 m length × 0.25 mm id × 0.25 ìm film thickness) was used with a initial temperature program of 80°C for 1 min; increased up to 200°C at 8°C/min and held for 2 min. and finally increased up to 260°C at 15°C/min and held at 260°C for 10 min. Nitrogen was used as the carrier gas at a constant flow of 1.0 ml/min. The samples were analyzed in split mode (1:20) at an injection temperature of 260°C and an EI source temperature of 230°C and scanned in the mass range from 50 *m*/*z* to 450 *m*/*z*.

Residual quantification analysis of Carbendazim by High performance liquid chromatography (HPLC) :

After every 14 day's interval the final concentration of Carbendazim was determined by High performance liquid chromatography (HPLC). The analysis of culture samples were carried out at Khadkeshwar oil mill Pvt. Ltd. Aurangabad. Degradation of carbendazim was monitored by measuring the reduction of the pesticide concentration in the cultures with a method of high performance liquid chromatography (HPLC). Water binary HPLC-1525 and detector - SERIES 200 UV/VIS was applied. One ml culture were collected and filtered through a 0.22µm syringe filter (Fisherbrandt) in order to remove the cells. Filtrates were stored at -20°C for further analysis. The analysis were conducted with a column Supelcosil™, C-18 (4.6 cm x 75 mm, 3.5 µm; SUPELCO) and at a wavelength of λ= 281 nm. A solution Methanol: Water (75:25) Ratio constituted the mobile phase. Fifty µl samples of filtrate were chromatographed in triplicates. The isocratic separation was carried out at a flow rate of 1ml/min; the quantity of pesticide in the sample was determined based on the peak absorption during the retention time obtained from the standard. The pesticide concentration in the sample was determined based on the standard curve. The metabolites of degraded sample of carbendazim were detected by Liquid Chromatography with Mass spectrophotometer from Dr. Analytical laboratory Poona(MS) India.

Residual quantification analysis of 2, 4-D by High performance liquid chromatography (HPLC):

After every 14 day's interval the final concentration of 2, 4-D was determined by High performance liquid chromatography (HPLC). The analysis of culture samples were carried out at Khadkeshwar oil mill Pvt. Ltd. Aurangabad. Degradation of 2,4-D was monitored by measuring the reduction of the pesticide concentration in the cultures with a method of high performance liquid chromatography (HPLC). Water binary HPLC-1525 and detector - SERIES 200 UV/VIS was applied. One ml culture were collected and filtered through a 0.22µm syringe filter (Fisherbrandt) in order to remove the cells and

fibers. Filtrates were stored at -20°C for further analysis. The analysis were conducted with a column Supelcosil™, C-18 (4.6 cm x 250 mm, 5 µm; SUPELCO) and at a wavelength of λ= 230 nm. A solution Methanol: Water (75:25) Ratio constituted the mobile phase. Fifty µl samples of filtrate were chromatographed in triplicates. The isocratic separation was carried out at a flow rate of 1ml/min; the quantity of pesticide in the sample was determined based on the peak absorption during the retention time obtained from the standard. The pesticide concentration in the sample was determined based on the standard curve. The metabolites of degraded sample of 2, 4-D were detected by Liquid Chromatography with Mass spectrophotometer from Dr. Analytical laboratory Pune.

The level of pesticides biodegradation (%) was calculated from the equation:

$$B = \frac{a-b}{a} \times 100$$

Where;

B= Biodegradation (%).

a=Concentration of pesticides in culture after t_0.

b= Concentration of pesticides in culture after t_{14}

The microorganisms isolated from the soil samples were identified by staining with lacto-phenol and cotton blue and were compared its morphology with standard literature. On the basis of morphological characterization the four fungal cultures were identified as *Trichoderma viride, Trichoderma koningii, Penicillium chrysogenum* and *Rhizopus stoloniferous*.

Biodegradation of Cypermethrin-25EC, Carbendazim and 2, 4-D :

Pesticide have made a great impact on human health, production and preservation of food, fiber and cash crops by controlling disease vector and by keeping in check many species of unwanted insect and plant. Pesticides are necessary to protect crop and losses may amount to about 45% of total food production worldwide but continuous use of these pesticides arising several problem regarding soil health as well as human health. Therefore a variety of physical and chemical methods are available to treat the soils contaminated with hazardous materials, but many of these physical-chemical treatments do not actually destroy hazardous compound but are bound in a modified matrix or transferred from one phase to another hence biological treatment is essential. It will help in the transformation of complex or simple chemical compounds into non hazardous forms.

However only few microorganisms are capable to pesticides degradation, therefore today's need to remediation of soils and detoxification of pesticides is very essential so Cypermethrin-25EC, Carbendazim (Bavistin) and 2,4-D which are most abundantly used as pesticides in worldwide therefore selected for degradation study. Degradation of these pesticides selected as microbial degradation and evaluation of microbial degraded residue using qualitative and quantitative analysis as Chemical oxygen demand, Gas chromatography with electronic capture detector, High performance liquid chromatography, Gas chromatography and mass spectrophotometer and Liquid Chromatography with mass spectrophotometer methods.

Table-1. Chemical oxygen demand of Cypermethrin microbial degraded residue and control after periodic day's intervals

Concen-trations	Days	Cont.	*T.v.*	%	*T.k.*	%	*P.c.*	%	*R.s.*	%	C.V.
50 mg/l	3	11033 ±15.57	9450 ±23.59	14.34	9600 ±9.433	12.98	9750 ±20.43	11.62	9800 ±16.51	11.17	0.2091
	7	10566 ±15.57	7333 ±1.248	30.59	7800 6.242	26.17	7766 ±2.06	26.50	7400 ±8.173	29.96	0.1431
	11	10766 ±3.775	4733 ±6.132	56.03	4866 ±5.191	54.80	5600 ±6.606	47.98	6033 ±3.402	43.96	0.1804
	14	10000 ±12.48	2200 ±6.242	78.00	2300 ±10.8	77.00	2933 ±6.132	70.67	3266 ±6.606	67.34	0.5007
100 mg/l	3	9066 ±5.191	17233 ±6.132	9.613	16700 ±2.359	12.40	17133 ±1.415	10.13	19033 ±3.775	0.173	0.0376
	7	19733 ±6.132	16000 ±10.28	18.91	16000 ±2.359	18.91	16066 ±6.606	18.58	16666 ±5.191	15.54	0.0762
	11	19533 ±8.491	10000 ±11.79	48.80	9866 ±2.831	49.49	10133 ±4.719	48.12	11833 ±5.663	39.42	0.1148
	14	19066 ±1.887	7400 ±3.303	61.08	7566 ±3.303	60.31	7466 ±6.132	60.84	7700 ±6.982	59.61	0.0829
150 mg/l	3	28966 ±2.359	25200 ±6.242	13.00	26266 ±1.415	09.32	25666 ±2.452	11.39	27100 ±6.572	06.42	0.0254
	7	29033 ±2.87	22166 ±1.701	23.65	22166 ±1.248	23.65	21800 ±3.303	29.91	23133 ±7.768	20.32	0.0309
	11	28666 ±4.032	19366 ±5.721	32.44	19633 ±6.982	31.51	19266 ±2.452	32.79	20000 ±4.194	30.23	0.0351
	14	28533 ±1.634	19266 ±2.87	32.47	16766 ±4.501	41.23	16700 ±6.947	41.47	19233 ±5.318	32.59	0.0520

Value expressed as mean of triplicates± S.E.M= Standard error of mean. C.V.= Coefficient variance of *T.v.- Trichoderma viride, T.k.- Trichoderma koningii, P.c.- Penicillium chrysogenum* and *R.s.- Rhizopus stoloniferous*.

Chemical oxygen demand of pesticides residue (COD) :

Cypermethrin-25EC :

During the experiment, a good correlation was established between Chemical Oxygen Demand removal and Cypermethrin degradation rates. It was observed that microorganisms showed degradation of Cypermethrin at different concentrations of 50mg/l, 100mg/l and 150mg/lin minimal minerals salt medium at 3rd, 7th, 11th and 14th day's intervals (Table-8, Fig.). The percentage decreased during the biodegradation

Cypermethrin following result was obtained. The four fungi selected for degradation study as *T. viride, T. koningii, P. chrysogenum* and *R. stoloniferous* among the *T. viride* had recorded maximum degradation potential at 50 mg/l 14.34%, 30.59%, 56.03% and 78.00%, at 100 mg/l 9.613%, 18.91%, 48.80% and 61.08% and at 150 mg/l 13.00%, 23.65%, 32.44% and 32.47% respectively. In *T. koningii* at 50mg/l it was 12.98%, 26.17%, 54.80% and 77.00%, at 100 mg/l 12.40%, 18.91%, 49.49% and 60.31% respectively and lastly at 150 mg/l 09.32%, 23.65%, 31.51% and 41.23% respectively. In *P. chrysogenum* at 50 mg/l 11.62%, 26.50%, 47.98% and 70.67%, at 100 mg/l 10.13%, 18.58%, 48.12% and 60.84% and at 150 mg/l 11.39%, 29.91%, 32.79% and 41.47%. Finaly *R. stoloniferus* at 50 mg/l 11.17%, 29.96%, 43.96% and 67.34% respectively, at 100 mg/l it was showed 0.173%, 15.54%, 39.42% and 59.61% respectively and at 150 mg/l showed very less degradation was recorded 06.42%, 20.32%, 30.23% and 32.59% respectively (Table-1).

Cypermethrin concentration detected by GC-ECD:

fungi *T. viride, T. koningii, P. chrysogenum* and *R. stoloniferous* were enriched and adapted by scale up process in minimal salt medium (MSM) containing Cypermethrin as a sole source of carbon and energy at varying concentration as 50mg/l, 100mg/l and 150mg/l respectively. The scale up process was carried out with successive frequent microbial sub-cultures from lower concentration to higher concentration of cypermethrin after 14 days time interval under continuous incubation at 30^{o} C with 160 rpm shaking speed.

The fungi *T. viride* had maximum degradation up to 66.06%, 58.59 % and 41.01% respectively than the control. In *T. koningii* showed 61.81%, 56.01% and 36.36% degradation respectively. In *P. chrysogenum* recorded 55.01%, 54.69% and 35.80% degradation respectively and finally least degradation was by R. *stoloniferous* up to 47.89%, 35.00% and 13.63% at 50 mg/l, 100 mg/l and 150 mg/l concentration of cypermethrin respectively (Table-2).

The comparative growth rate of *Trichoderma viride, Trichoderma koningii, Penicillium chrysogenum* and *Rhizopus stoloniferous* recorded dry mycelial weight after 14 days duration. In that *T. viride* had more dry mycelial weight than *T. koningii, P. chrysogenum* and *R. stoloniferus*; it means that *T. viride* growth rate was faster and used Cypermethrin as source of carbon and energy.

Carbendazim (Bavistin): COD

The Carbendazim degradation studies were carried out at three concentrations of 100mg/l, 200mg/l and 300mg/l in minimal minerals salt medium at 3rd, 7th, 11th and 14th day's intervals by scale up procedure with selected microorganisms. The percentage decreased in Chemical oxygen demand measured during the biodegradation of carbendazim showed following results.

Fungi *T. viride* showed at 100 mg/l 20.00%, 39.52%, 55.13% and 56.99%, at 200 mg/l 14.42%, 23.40%, 34.01% and 38.83% and at 300 mg/l 02.64%, 03.09%, 07.82% and 12.31% respectively. In *T. koningii* at 100 mg/l recorded 23.67%, 35.13%, 54.45% and 56.65%, at 200 mg/l 15.10%, 26.60%, 36.72%

and 37.82% and at 300 mg/l 01.72%, 06.25%, 07.47% and 11.73% degradation respectively. In *P. chrysogenum* at 100 mg/l 16.34%, 34.45%, 53.42% and 55.63%, at 200 mg/l 09.64%, 18.18%, 34.01% and 35.40% and at 300 mg/l 04.02%, 05.85%, 08.51% and 11.84% respectively. Finally *R. stoloniferus* at 100 mg/l showed 16.34%, 34.63%, 53.07% and 55.63%, at 200 mg/l 12.26%, 16.50%, 29.44% and 26.95% and lastly at 300 mg/l showed least as 00.02%, 03.44%, 06.55% and 10.16% respectively (Table-3).

Carbendazim Concentration detected by HPLC :

The Fungus *Trichoderma viride, Trichoderma koningii, Penicillium chrysogenum* and *Rhizopus stoloniferous* had been enriched and adopted by scale up process in minimal minerals salt medium (MSM) containing Carbendazim as sole source of Carbon and energy at different concentrations as 100 mg/l, 200 mg/l and 300 mg/l respectively.

The HPLC data illustrate that during 14 days incubation period the *T. koningii* recorded 65.11%, 55.98% and 33.22%, in *P. chrysogenum* 59.93%, 29.05% and 17.59% degradation respectively. In *T. viride* showed 47.84%, 42.11% and 27.12% respectively. Finally the fungi *R. stoloniferous* showed least degradation as 43.78%, and 18.99%

Table-2. GC-ECD Analysis of Cypermethrin microbial degraded residue

Pestcides Conc.	M.O.	Area of Sample	Area of Std.	Dilution factor	Concentration mg/l	Degradation %
50mg/L	Control	25.702	166.394	500	3.09	00.00
	T.v.	180.812	137.348	20	1.05	66.01
	T.k.	48.886	166.394	100	1.18	61.81
	P.c.	101.237	291.892	100	1.39	55.01
	R.s.	117.234	291.892	100	1.61	**47.89**
100 mg/L	Control	47.739	291.892	1000	6.54	00.00
	T.v.	37.184	273.71	500	2.72	58.59
	T.k.	240.707	166.394	50	2.89	56.01
	P.c.	722.474	291.892	100	2.97	54.79
	R.s.	70.847	273.71	300	3.11	**35.00**
150 mg/L	Control	61.724	273.71	1000	9.02	00.00
	T.v.	77.715	291.892	500	5.32	41.01
	T.k.	196.328	273.71	200	5.74	36.36
	P.c.	211.432	291.892	200	5.79	35.80
	R.s.	53.278	273.71	1000	7.79	**13.63**

T.v.- Trichoderma viride, T.k.- Trichoderma koningii, P.c.- Penicillium chrysogenum and *R.s.- Rhizopus stoloniferous*.

Table-3. Chemical oxygen demand of Carbendazim microbial degraded residue and control after periodic day's intervals

Concen-trations	Days	Cont.	*T.v.*	%	*T.k.*	%	*P.c.*	%	*R.s.*	%	C.V.
100 mg/l	3	10000 ±5.564	8000 ±10.8	20.00	7633 ±4.719	23.67	8366 ±2.359	16.34	8400 ±4.927	16.00	0.1275
	7	9867 ±3.775	5967 ±3.863	39.52	6400 ±9.421	35.13	6467 ±1.415	34.45	6450 ±11.79	34.63	0.1601
	11	9733 ±1.701	4367 ±2.947	55.13	4433 ±3.402	54.45	4533 ±6.803	53.42	4567 ±3.094	53.07	0.1461
	14	9766 ±6.947	4200 ±6.242	56.99	4233 ±4.325	56.65	4333 ±6.132	55.63	4333 ±5.663	55.63	0.2217
200 mg/l	3	19866 ±5.663	17000 ±8.225	14.42	16866 ±6.502	15.10	17966 ±5.797	09.64	17433 ±2.947	12.26	0.0558
	7	19800 ±7.26	15166 ±2.057	23.40	14533 ±2.831	26.60	16200 ±5.913	18.18	16533 ±1.701	16.5	0.0545
	11	19700 ±9.04	13000 ±8.815	34.01	12466 ±6.132	36.72	13000 ±8.173	34.01	13900 ±4.719	29.44	0.0991
	14	16566 ±2.452	10133 ±4.247	38.83	10300 ±7.595	37.82	10700 ±2.452	35.40	12100 ±4.194	26.95	0.0644
300 mg/l	3	28966 ±4.647	28200 ±5.913	02.64	28466 ±4.501	01.72	27800 ±10.62	04.02	28100 ±6.947	00.02	0.0373
	7	29033 ±5.36	28133 ±1.415	03.09	27216 ±3.775	06.25	27333 ±3.745	05.85	28033 ±4.927	03.44	0.0250
	11	28966 ±5.741	26633 ±2.162	07.82	26800 ±3.686	07.47	26500 ±4.032	08.51	27000 ±4.194	06.55	0.0206
	14	28700 ±5.721	25166 ±2.057	12.31	25333 ±3.269	11.73	25300 ±6.947	11.84	25783 ±3.303	10.16	0.0267

Value expressed as mean of triplicates± S.E.M= Standard error of mean. C.V.= Coefficient variance of *T.v.- Trichoderma viride, T.k.- Trichoderma koningii, P.c.- Penicillium chrysogenum* and *R.s.- Rhizopus stoloniferous*.

respectively as compared to control. After 7 days incubation the fungi *T. viride, T. koningii,* and *R. stoloniferous* showed color change of media from colorless to dark brown while *P. chrysogenum* showed green color in minimal minerals medium (Table-4).

In Carbendazim *T. viride* and *T. koningii* recorded maximum mycelia dry weight than the *Penicillium chrysogenum* and *Rhizopus stoloniferous*, therefore *T. viride* had highest growth rate in the presence of Carbendazim. In fungi *T. koningii* showed good potential for Carbendazim degradation as 65.11%, 55.98% and 33.22% at 100 mg/l, 200 mg/l and 300 mg/l respectively (Table-11). But Itamar De Melo *et al*.,[5] reported that strains of *T. harzianum* had Carbendazim degradation ability.

Table-4. HPLC Analysis of Carbendazim microbial degraded residue.

Pestcides Conc.	Microorganiks	Height (μV)	Retention time (Min.)	Area	Concen-tration mg/l	Degra-dation %
100mg/L	Standard Cabendazim	274035	1.068	1530626	-	-
	Control	82732	1.087	452024	14.76	00.00
	T.v.	46665	1.080	254099	7.701	47.84
	T.k.	28844	1.090	157702	5.151	65.11
	P.c.	43231	1.081	235764	5.916	59.93
	R.s.	33079	1.096	181107	8.30	43.78
200 mg/L	Standard Cabendazim	274035	1.068	1530626	-	-
	Control	156934	1.086	863410	28.204	00.00
	T.v.	91879	1.087	499748	16.325	42.11
	T.k.	70288	1.085	380060	12.415	55.98
	P.c.	111576	1.076	612484	20.008	29.05
	R.s.	93711	1.091	510467	16.675	40.87
300 mg/L	Standard Cabendazim	274035	1.068	1530626	-	-
	Control	226566	1.077	1303875	42.592	00.00
	T.v.	168526	1.076	950286	31.042	27.12
	T.k.	156899	1.072	870902	28.44	33.22
	P.c.	190337	1.081	1074679	35.10	17.59
	R.s.	186621	1.080	1056380	34.50	18.99

T.v.- Trichoderma viride, T.k.- Trichoderma koningii, P.c.- Penicillium chrysogenum and *R.s.- Rhizopus stolanifer*.

2, 4-D: Chemical Oxygen Demand :

2, 4-D herbicide degradation in laboratory condition were studied by using biological source as microorganisms. The selected microorganisms were used in 2, 4-D degradation study at 300 mg/l, 600 mg/l and 900 mg/l concentrations in minimal minerals salt medium at 3rd, 7th, 11th and 14th intervals. The percentage decreased in COD was measured during the biodegradation of 2, 4-D showed following results.

Fungi: *P. chrysogenum* showed degradation at 300 mg/l 23.68%, 36.64%, 57.95% and 72.16% respectively, at 600 mg/l 15.16%, 36.50%, 58.03% and 59.39% and 900 mg/l 04.08%, 12.23%, 18.43% and 24.05% degradation respectively. In *T. koningii* at 300 mg/l recorded 20.67%, 26.71%, 53.40% and 67.03%, at 600 mg/l 11.17%, 29.84%, 52.46% and 54.69% and at 900 mg/l showed 02.87%, 07.78%, 09.33% and 14.70% respectively. In *T. viride* at 300 mg/l showed 19.17%, 29.39%, 54.62% and 63.01%, at 600 mg/l 11.12%,

30.16%, 50.32% and 60.73% and at 900 mg/l 04.96%, 11.90%, 17.18% and 24.17% removal of 2, 4-D respectively and lastly *R. stoloniferus* showed least 2, 4-D degradation, at 300 mg/l 16.53%, 30.15%, 50.00% and 59.71%, at 600 mg/l showed 11.12%, 31.42%, 49.18% and 50.84% and in last 900 mg/l it showed very less degradation as 02.09%, 05.78%, 07.84% and 11.40% respectively (Table-5).

2, 4-D detection by HPLC :

The eight cultures of Bacteria and fungi were used for 2, 4-D herbicide degradation by scale up process and evaluating residue by High performance liquid chromatography (HPLC). The cultures were incubated for two weeks at 30^{0}C in 100 ml minimal mineral salt medium containing 2, 4-D at 300 mg/l, 600 mg/

Table-5. Chemical oxygen demand of 2, 4-D microbial degraded residue and control after periodic day's intervals.

Concen-trations	Days	Cont.	*T.v.*	%	*T.k.*	%	*P.c.*	%	*R.s.*	%	C.V.
300 mg/l	3	8866 ±3.303	7166 ±6.132	19.17	7033 ±4.325	20.67	6766 ±3.863	23.68	7400 ±2.87	16.53	0.0918
	7	8733 ±2.831	6166 ±3.686	29.39	6400 ±4.194	26.71	5533 ±4.114	36.64	6100 ±6.023	30.15	0.1205
	11	8800 ±2.87	4033 ±0.933	54.62	4100 ±4.789	53.40	3700 ±5.721	57.95	4400 ±4.501	50.00	0.1438
	14	9100 ±4.032	3366 ±3.303	63.01	3000 ±3.863	67.03	2533 ±1.634	72.16	3666 ±1.701	59.71	0.1647
600 mg/l	3	20666 ±0.943	18366 ±2.162	11.12	18233 ±2.831	11.17	17533 ±3.563	15.16	18366 ±3.303	11.12	0.0327
	7	21000 ±5.36	14666 ±2.627	30.16	14733 ±2.452	29.84	13333 ±3.303	36.50	14400 ±3.563	31.42	0.0392
	11	20333 ±2.057	10100 ±4.194	50.32	9666 ±2.452	52.46	8533 ±2.831	58.03	10333 ±2.497	49.18	0.0488
	14	19866 ±4.927	9400 ±5.564	52.68	9000 ±3.686	54.69	8066 ±1.415	59.39	9766 ±5.797	50.84	0.0223
900 mg/l	3	30200 ±4.501	29666 ±2.947	01.76	29333 ±1.415	02.87	28966 ±6.687	04.08	29566 ±1.701	02.09	0.0175
	7	29966 ±1.415	27500 ±3.863	08.22	27633 ±2.057	07.78	26300 ±3.863	12.23	28233 ±1.415	05.78	0.0250
	11	29300 ±5.721	27166 ±2.87	07.28	26566 ±2.627	09.33	23900 ±2.87	18.43	27000 ±4.647	07.84	0.0289
	14	29233 ±2.497	24866 ±5.442	14.93	24933 ±3.402	14.70	22200 ±4.194	24.05	25900 ±5.104	11.40	0.0250

Value expressed as mean of triplicates± S.E.M= Standard error of mean. C.V.= Coefficient variance of *T.v.- Trichoderma viride, T.k.- Trichoderma koningii, P.c.- Penicillium chrysogenum* and *R.s.- Rhizopus stoloniferous*.

l and 900 mg/l concentrations at 160 rpm shaking speed.

Fungi *P. chrysogenum* 73.19%, 71.34% and 09.44% respectively. Followed by *T. viride* 59.99%, 59.47% and 04.83% respectively The finally least degradation recorded by *R. stoloniferous* and *T. koningii* 59.11% 47.87%, 2.734 and 55.72%, 52.82%, 12.37% respectively (Table-6). The *P. chrysogenum* first fungi for 2, 4-D degradation was reported by Tatiane M. Silva[9], he also reported Among 25 isolated strains, five presented major degrading potential, and were identified as *Acinetobacter* sp, *Serratia marcescens, Stenothrophomonas maltophilia*, *Flavobacterium* sp and *Penicillium* sp.

Omar[7] identified thirteen fungal species isolated from pesticides treated soil and observed their ability to mineralize and degrade three organophosphate insecticides as a phosphorus source. It is reported that out of thirteen fungal species, only some *Aspergillus* species viz., *A. niger*, *A. tamarii*, *A. terreus* and *Trichoderma harzianum* utilized pesticides as a nutrient source.

Table-6. HPLC Analysis of 2, 4-D microbial degraded residue.

Pestcides Conc.	Microorganiks	Height (μV)	Retention time (Min.)	Area	Concen-tration mg/l	Degra-dation %
300mg/L	Standard 2, 4-D	306666	2.085	284677	-	-
	Control	12358	2.053	64331	11.298	00.00
	T.v.	3898	2.090	25762	4.520	59.99
	T.k.	4346	2.096	28483	5.002	55.72
	P.c.	2634	2.109	17244	3.028	73.19
	R.s.	3900	2.113	26302	4.619	59.11
600 mg/L	Standard 2, 4-D	306666	2.085	284677	-	-
	Control	14745	2.158	105452	18.52	00.00
	T.v.	4979	2.203	42734	7.505	59.47
	T.k.	6132	2.196	49748	8.737	52.82
	P.c.	3390	2.264	30216	5.307	71.34
	R.s.	5667	2.217	54971	9.654	47.87
900 mg/L	Standard 2, 4-D	23110	2.446	404625	-	-
	Control	17304	2.348	245947	30.391	00.00
	T.v.	16055	2.438	234056	28.922	04.83
	T.k.	15298	2.459	215521	26.63	12.37
	P.c.	16189	2.464	222769	27.52	09.44
	R.s.	15988	2.453	239227	29.56	02.73

T.v.- Trichoderma viride, T.k.- Trichoderma koningii, P.c.- Penicillium chrysogenum and *R.s.- Rhizopus stoloniferous*.

Microbiological degradation is one of the most important processes that determine the fate of pesticides in the environment. Microorganisms are highly effective in transforming organic pollutants and modifying their structure and toxic properties; also, they can completely mineralize organic compounds to non-organic products[14].

Occurrence of a "new" foreign organic substance in the environment usually leads to selection of certain sensitive microorganisms, while the remainder adapts to the new environmental conditions. In the course of the adaptation, microorganisms initiate enzymatic processes that enable them to utilize compounds that are not the products of their own metabolic transformation as energetic and building substrates (microbiological metabolism), or these substances are incorporated in the metabolic cycle of microbial Cells and are not used as an energy source (microbiological metabolism)[11].

The overall finding suggested that the technique for the degradation of commonly used Cypermethrin, Carbendazim and 2, 4-D pesticides by fungal cultures may be feasible and reasonable treatment option for the removal of pesticides from soil and water as bioremediation observed only in presence of acclimated microorganisms has been designed to develop the technique as pilot scale up for *in-vitro* in minimal minerals salt medium and cow-dung slurry with microbial consortium for *in-vivo* in soil. The treatment unit has been designed to develop the technique for bioremediation of surface soil containing pesticides by monitoring and maintaining environmental parameters under stimulated conditions.

References :

1. Brinch U.G., F. Ekuland and C.S. Jacobesen (2002). *Applied and Environ. Microbiol. 68* (4): 1808-1816.
2. Cooney N.L, and D. W. Levine. (1972). *Adv. Appl. Microbiol. 15:* 337-365.
3. Cserjesi, A.J. (1967). *Can. J. Microbiol.*, *13:* 1243-1249.
4. Curtis, R.F., D.G. Land and N.M. Griffeths (1972). 2,3,4,6-tetrachloanisole association with musty taint in chickens and microbiological formation. *Nature*, *235:* 223-224.
5. Itamar Soares De Melo, Celia Maria Maganhotto De S. Silva and Jane L. Faull. (2010). *Pesticides: r. ecotoxicol. e meio ambiente, Curitiba. 20:* 1-6.
6. Joseph C. Gilman. (2001). A Manual of soil fungi. Biotech books, Delhi (India).
7. Omar, S.A. (1998). *Appl. Biochem. Microbiol., 40:* 1-17.
8. Siddiqui Zamin Shaheed and Arif-Uz-Zaman. (2004). *L. Pak. J. Bot. 36*(3): 577-582.
9. Surekha R.M., P.K.L. Lakshmi, D. Suvarnalatha, M. Jaya, S. Aruna, K. Jhothi, G. Narsimha and K. Venkateswarl (2008). *Afr. Journal of Microbiol. Res. 2:* 26-31.
10. Tatiane M. S., I. S. Maria, M. M. Andre, D. A. Fabiana, A. V. P. Sonia, R. F. Paulo, D. C. Marcelo, C. Emanuel, I.B.C. Paulo and P. Marcos (2007). *Brazilian journal of Microbiology. 38:* 522-525.

11. Trivedy R.K. and P.K. Goel (1984). Chemical and biological methods for water pollution studies. Environmental publication, Karad (India).
12. Warren N., I.J. Allan, J.E. Carter, W.A. House and A. Parker. (2003). *Appl. Geochem., 18:* 159.
13. White, R.B. and S.D. Aust. (1994). *AAPg Bulletin, 78*(8): 1335-1341.
14. Zipper C.H., K. Nickel, W. Angst and H.P.E. Kohler. (1996). *Appl. Environ. Microbiol. 62 :* 4318-4322.