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In situ Biodegradation of Endosulfan, Imidacloprid, and Carbendazim Using Indigenous Bacterial Cultures of Agriculture Fields of Uttarakhand, India

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***Abstract*—**In the present study, presence of endosulfan, imidacloprid, carbendazim, in the soil /vegetables/cereals and water samples was observed in agriculture fields of Uttarakhand. In view of biodegradation of these pesticides, 9 bacterial isolates were recovered from the soil samples of the fields which tolerated endosulfan, imidacloprid, carbendazim from 100 to 200 µg/ml. Three bacterial consortia used for *in vitro* bioremediation experiments were consisted of 3 bacterial isolates for carbendazim, imidacloprid and endosulfan, respectively. Maximum degradation (87 and 83%) of α and β endosulfan respectively was observed in soil slurry by consortium. Degradation of Imidacloprid and carbendazim under similar conditions was 88.4 and 77.5% respectively. FT-IR analysis of biodegraded samples of pesticides in liquid media showed stretching of various bonds. GC-MS of biodegraded endosulfan sample in soil slurry showed the presence of nontoxic intermediates. A pot trial with Bacterial treatments lowered down the uptake of pesticides in onion plants.

***Keywords*—**Biodegradation, carbendazim, consortium, Endosulfan.

I. Introduction

PLANT protection by means of synthetic chemicals/ pesticides has become one of the essential components of agriculture to maintain higher food production to feed millions of population. Besides agriculture, large amount of pesticides is used for urban plantation, hygienic handling and storage, wood preservation, to check mold growth in paper industries and in food items. Apart from socioeconomic upliftment of farming communities by the use of pesticides, strong disadvantages also came into existence. Out of total pesticides applied only 0.1-2% reaches to the target organisms [1] and rest enters into different parts of environmental components causing adverse effect all around. The indiscriminate use of agrochemicals has caused serious environmental problems [2]. The behavior of a pesticide in the environment depends on its stability, physico-chemical properties of the soil, microbial diversity and rhizospheric activities. The residual effect of pesticides on human includes carcinogenicity, mutagenicity, reproductive toxicity and other health problems [3]. In this study, presence of endosulfan, carbendazim and imidacloprid has been reported in the agricultural soil of Uttarakhand state, hence attempt to biodegrade these pesticides has been undertaken.

Endosulfan, an organochlorine insecticide had been used to control insects, like colorado beetle, flea beetle, cabbage worm, aphids and leaf hopper on a wide range of crops, including cereals, cotton, coffee, fruits, oil seeds and vegetables for more than 5 decades [4]. In India, it is mainly used in rice, cotton and tea. Organochlorine pesticides are highly persistent and resist biodegradation because of their cyclic nature. Presence of a reactive cyclic sulphite diester group in endosulfan makes it moderately persistent as compared to other organochlorines [5].Technical endosulfan is a mixture of α and β isomers. Thermodynamically α is more stable than β. Over all dissipation of the pesticide depends upon volatilization, alkaline hydrolysis and photodecomposition, besides the nature of fertilizer, crop, temperature, rain and biotic factors. Endosulfan accumulates in soil and water and ultimately affects human health. It is extremely toxic to aquatic fauna and may provoke chronic symptoms like testicular and prostate cancer breast cancer and sexual abnormality, genotoxicity and neurotoxicity in numerous mammalian species [6]. Intermediate products of endosulfan have been detected in soils, sediments, surface water and foods with a half-life of 3-6 months [7]. Microbes have the ability to degrade pesticides either completely or partially and this process is affected by sorption and transport of pesticides in soil. Indigenous microorganisms of the soil are reported to oxidize endosulfan to endosulfan sulfate. Different bacteria and fungi, isolated from pesticide contaminated sites have the capability to degrade endosulfan [5]. Biodegradation of endosulfan can occur either by oxidation to form toxic intermediate like endosulfan sulfate or by hydrolysis to form the less toxic endosulfan diol [8].The compound breaks down into endosulfan sulfate, endosulfan diol and endosulfan furan.

Imidacloprid belongs to a new class of neonicotinoid insecticides and shows reduced toxicity in mammals than insects compared to organophosphates and carbamates. It is effective against sucking insects, chewing insects and fleas of

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domestic animals [9]. Its characteristics of less soil sorption and high leaching capability make it potential contaminant of surface and underground water [10]. Vegetation increased the rate of dissipation of imidacloprid yielding a range of half lives from 42 to 129 days as compared to without vegetation and was 180 days [11]. Metabolites of imidacloprid found in the soil samples are 6- chloronicotinic acids, two cyclic urea, olefinic cyclic nitroguanidine, a cyclic guanidine and nitroso and nitro derivatives. Sorption of imidacloprid generally increases with soil organic matter and depends on pesticide concentration. Anhalt et al. [12] reported the biodegradation of imidacloprid by *Leifsonia* strain PC-21.Carbendazim is a fungicide of major concern due to its effect on endocrine followed by hormone disruption. It is very toxic to fish and aquatic life. Main biodegraded product of carbendazim is 2-aminobenzimidazole which is further decomposed by microbial activity. Xu et al [13] showed biodegradation of carbendazim using *Rhodococcus* sp. dil-6 in M9 medium with an average rate of 55.56 $mg^{-1}d^{-1}$.Role of rhizospheric region in biodegradation of pesticides has been reported by several authors.

II. Materials and Methods

A. Field Survey and Collection of Pesticide Contaminated Samples

Rhizospheric or subsurface soil samples , collected at a depth of 15-30cm in sterile polythene bags from the agriculture fields of Udham Singh Nagar and Nainital having history of regular pesticide application were used for the isolation of pesticide degrading microorganisms and residual analysis of the pesticides.

B. Residual Analysis of Pesticides in Soil Samples

Residual analysis of the pesticides in the contaminated soil samples was performed according to modified method of Anastassiades et al. [14]. Extracted pesticides were analyzed by GC/HPLC.

C. Isolation of Pesticide Degrading Bacteria

Diluted soil samples (10^{-5} -10^{-6}) were pour plated in nutrient agar to get discrete bacterial colonies. Morphological distinct colonies were purified by repeated streaking on pesticide supplemented media and pure cultures were maintained in nutrient agar slants.

D. Chemicals and Reagents

Technical grade pesticides were kindly provided by Department of Chemistry, of our University. All the chemicals used in this study were of analytical grade. For screening of carbendazim and imidacloprid degrading bacteria MSM (minimal salt medium) was used whereas NSM (non sulfur medium with 1% glucose) was used for the isolation of endosulfan degrading bacteria. Biodegradation studies of the pesticides were performed in minimal medium.

E. Maximum Tolerance Level of Pesticides in Selected Bacterial Isolates

Different sets of sterilized minimal broth (25 ml), supplemented with pesticide(s) @10 to 210 ppm were inoculated with 100 µl active bacterial culture(s) and incubated at 28±2^{o}C for 72 h at 120 rpm. Uninoculated flask served as control. Bacterial growth was measured by taking absorbance at 600 nm [15]. The concentration of pesticide that supported maximum growth was observed.

F. Molecular Characterization of Pesticide Degrading Bacteria

Nine Bacterial isolates showing maximum growth at highest concentration of pesticide(s) were characterized on the basis of DNA sequencing.

G. Genomic DNA Extraction

Genomic DNA of selected bacterial isolates was extracted according Bazzicalupo and Fani [16].

H. Amplification of 16S rDNA

Amplification of genomic DNA using universal primers was carried out in a thermal cycler (PTC-200 model, M.J. Research).The amplified product was sequenced by Chromous Biotech. Bangalore, India. On the basis of sequence analysis, bacterial strains were identified using NCBI data base. Closest BLAST match was used for phylogenetic analysis.

I. Preparation of Bacterial Inoculum and Consortium

Potent bacterial isolate(s) were grown in 50 ml of nutrient broth containing respective pesticides @ 20ppm) at 28±ºC for 24h to obtain an OD_{600} 0.6 /10^5 to 10^6 CFU/ml. To develop a consortium, individual bacterial isolate(s) were streaked on nutrient agar plate to find their compatibility. GB20, GB21 and GB61 were used as carbendazim degrading consortium, similarly GB5, GB35 and GB78 were used as imidacloprid degrading consortium. Consortium of GB69, GB72 and GBA was used for endosulfan degradation.

J. Biodegradation of Pesticides in Minimal Medium

The experiment was performed in triplicate. Fifty ml of MSM (mineral salt medium) or NSM broth taken in a 100 ml flask was supplemented with pesticide (100ppm) and inoculated with 1 ml of 24 hr old bacterial culture. One ml aliquot of the broth was taken from all the flasks on 0, 10, 15 and 20th day for extraction. Uninoculated flasks, spiked with pesticide(s) acted as control. Quantification of pesticide was done by GC and HPLC.

K. Biodegradation of Pesticides in Soil Microcosm

Fifty g of autoclaved soil was taken in a 100 ml flask. Pesticide (100ppm) was added to all the sets, to which 1 ml of 24 hr old bacterial culture was also added. One g of soil was taken from all the flasks separately on 0, 10, 15 and 20th day for extraction. Uninoculated flasks spiked with pesticides acted as control. The experiment was performed in triplicate. Extraction and quantification of pesticide was done as described elsewhere.

L. Biodegradation of Pesticides in Soil Slurry

Fifty g autoclaved soil was taken in a 100 ml flask, to which 20ml of minimal medium was added. After addition of pesticide (100ppm) to all the sets, 1 ml of 24 hr old bacterial culture was added and mixed properly. One ml of soil slurry was taken from the individual flask separately on 0, 10, 15 and 20^{th} day for extraction. Uninoculated flasks spiked with pesticides acted as control. Quantification of pesticide was done by GC and HPLC. Samples were also analyzed by FTIR and GCMS [17].

M. In situ Study of Pesticide Remediation with Potent Isolates

To analyze the efficiency of selected bacterial isolates in pesticide removal from the soil under natural conditions, a pot experiment was conducted on onion. Experiment was conducted in triplicate in the departmental net house in unsterilized soil. Two concentrations of pesticides (50 and 100 mg/ Kg soil) were taken. The treatments were: (1) Control 3: (soil+pesticide), (2) Control 3: (soil+pesticide+plant), (3) 9 treatments of nine bacteria and three pesticides (soil+pesticide+plant+bacteria), (4) treatments of 3 consortia (soil+pesticide+plant+consortium). Total 36 treatments were included with 18 sets of 50 mg /Kg pesticide and 18 sets of 100 mg /Kg pesticide.

N. Seedlig Sterilization and Sowing

Roots of onion seedlings, obtained from Vegetable Research Centre of the University were surface sterilized with $HgCl_2$ (0.2%) for 5 min. and washed thoroughly with sterile distilled water. For bacterial treatments, seedlings were dipped in bacterial culture(s) + 1% CMC and transferred to plastic pots @ 4 seedlings per pot. Pots were watered regularly.

O. Sample Collection and Analysis

Onion plants were harvested after 30 days and analyzed for parameters like shoot height, root height and plant dry weight. Total uptake of pesticide in the plant was estimated by GC/HPLC after extraction. Soil samples from all the pots were also collected at the time of harvesting and analyzed for residual pesticide(s).

P. Analytical Procedures

Residual pesticide was extracted by adding 5mL of culture broth / 5 g of soil to 20 ml acetone in a flask and shaken for 1 h. Mixture was then filtered using Buchner funnel and obtained residue was washed thoroughly with 10 ml acetone and filtered. Filtrate was collected in a round bottom flask and evaporated to dryness in a rotary flash evaporator at 50ºC. The residue was dissolved in n-hexane and filtered by 0.2 µ filter. Gas Chromatography (Chemito, Ceres 800 plus, packed column 10% SE 30, ^{63}Ni ECD) was used for endosulfan analysis. Nitrogen acted as carrier and make-up gas. The oven, injector and detector temperature were programmed at 180ºC, 260ºC and 300ºC respectively. Samples of imidacloprid and caebendazim were analyzed using HPLC. Samples of soil slurry were also analyzed by FTIR at Department of Biophysics, of the University. Facilities for GC-MS were provided by Tezpur University.

III. Results and Discussion

A. Survey and Sample Collection

A survey of agriculture fields of Distt. Udham Singh Nagar and Nainital was done in 2012-2013 to collect soil samples. Soil samples showed the presence of carbendazim (0.03µg/g – 0.22µg/g), imidacloprid (.02-.1 µg/g)and endosulfan (0.01-0.32 µg/g).According to environmental quality standards of the soil, 3 level of soil contamination are proposed: slightly polluted soil (0.05-0.5 mg Kg^{-1}, moderately polluted (0.5- 1 mg Kg^{-1}) and heavily polluted soil with >1 mg Kg^{-1} [18].On comparing the residual level of pesticides in the soil samples of the present study with reported values, each soil sample showed little pollution. Nainital soil did not show the presence of any pesticide; however, traces of imidacloprid, chlorpyriphos were reported from Haldwani, where most of the samples were taken from vegetable fields. The levels of α-endosulfan ranged from 0.1 mg to 29 mg/kg, β-endosulfan from 0.1-167 mg/kg, and endosulfan sulphate from 0.12-187 mg/kg in soil samples collected from different regions of Karnataka and Kerala [19].Anhalt et al. [12] have reported degradation of imidacloprid with the help of a soil bacteria (*leifsonia*) and the products were imidacloprid guanidine and imidacloprid urea by HPLC and LC-MS analysis.

B. Isolation and Screening of Pesticide Degrading Bacteria

A sum of nine bacterial isolates (3 each for endosulfan, carbendazim and imidacloprid) were recovered from the contaminated soil (Table II). GB69, GB72, GBA were selected for their ability to grow on non sulphur medium (NSM) supplemented with endosulfan as a sulphur source. Three bacterial isolates: GB20, GB21, GB61 grew on carbendazim supplemented minimal medium. Similarly GB5, GB78 and GB35 utilized imidacloprid as a carbon source in minimal medium. Kataoka et al. [17] isolated endosulfan degrading fungus (*Mortierella* species), from the soil contaminated with organochlorines. Zhang et al. [20] isolated a carbendazim degrading *Rhodococcus erythropolis* djl 11 from contaminated soil by enrichment method. The organism degraded carbendazim @ 333.33 mg L^{-1} d^{-1} and produced two major metabolites namely 2- aminobenzimidazole and 2-hydroxybenzimidazole. Selective enrichment techniques have also been used for the isolation of γ-HCH degrading bacteria [21]. Pandey et al. [22] have isolated lindane degrading *Gordonia* spp., from pesticide contaminated soil of CRC, Pantnagar. *Brevundimonas* sp. MJ15 (SP-1) was enriched and isolated as imidacloprid degrading bacteria from agricultural soil with a history of imidacloprid exposure by Shetti and Kaliwal [23].Isolation of chlorpyrifos degrading *Pseudomonas* and *Bacillus* spp. from contaminated soil was reported [24]

C. Maximum Tolerance Level for Pesticide

Growth of nine bacterial isolates (O.D at 600 nm) increased gradually with increase in their respective pesticide concentration. Bacterial isolates namely GB20, GB21 and

GB61 tolerated carbendazim upto 130-150 µg/ml. GB69, GB72 and GBA tolerated 130-172 µg/ml of endosulfan. GB5, GB78 and GB35 grew on imidacloprid upto 110-150 µg/ml (Table II). According to Kale et al. [15] growth of *Azotobacter chroococcum* was not affected at 5 µg/ml of carbofuran and carbaryl but inhibited at higher concentrations of the same insecticides. Okeke et al. [25] reported 10-200 mg/L lindane degradation/utilization by *Pandoraea* sp. Decline in growth of *Pseudomonas* strains at 30 µg/ml and higher lindane concentrations was reported by Nawab et al. [26]

D.Molecular Characterization of Pesticide Degrading Bacteria

BLAST analysis of 16S rDNA sequences of pesticide degrading bacteria showed 99-100% homology with *Bacillus, Pseudomonas, Exiguobacterium, Microbacterium, Achromobacterium,* and *Stenotrophomonas* sp**.** (Table III).

E. Pesticide Biodegradation in Soil

After 20 days of pesticide application, minimum amount of imidacloprid, endosulfan and carbendazim was observed in soil microcosm inoculated with their respective consortium **(**Table IV). Maximum degradation of carbendazim was observed by consortium (84.8%) followed by GB61 (83.2%). Bacterial treatments enhanced carbendazim degradation from 17.2 to 84.8%. Similarly, highest amount of residual endosulfan isomers was observed in control, and addition of bacterial isolates reduced endosulfan concentration after 20 days. Pattern of endosulfan degradation was: consortium (84.6%) > GB72 (82.6%) > GB69 (80%) > GBA (79.6%) > control (24.2%) for α-endosulfan; for β-endosulfan the pattern was, consortium (81.3%) > GB72 (78.3%) > GB69 (76.3%) > GBA (74%) > control (20%). In imidacloprid, consortium showed 69% degradation as compared to control showing only 15% degradation. GB78 showed 64.8% degradation of imidacloprid in 20 days. Amount of imidacloprid in minimal medium in the presence of GB5, GB35, GB78 and consortium decreased from 100 to 37%, 38%, 35% and 31% respectively at the end of 20th day, however in control 81% imidacloprid remained undegraded after 20th day **(**Table IV). Tian and Chen [27] reported complete biodegradation of carbendazim by *Trichoderma* after 6 days in a soil remediation study. Kumar and Philip [28] reported that endosulfan degradation efficiency of the sandy soil in aerobic condition was 31.5±0.23%, whereas, in anaerobic condition it was 32.57±0.18%**.** Addition of bacterial cultures enhanced degradation to 90 and 78% in soil A and B, as compared to uninoculated soil showing only 20% and 38% degradation of endosulfan respectively [7]. In the inoculated soil samples, besides enhanced degradation of endosulfan, degradation of endosulfan diol was also observed. Accumulation of endosulfan sulfate, (a metabolite formed primarily by fungal activity) was not observed during degradation of endosulfan [7]. Degradation of endosulfan in soil microcosm by mixed and individual cultures (*Ochrobacterum* sp., *Arthrobacter* sp., and *Burkholderia* spp.) was studied in sandy loam agricultural soil by Kumar *et al*. [29]**.** Degradation of endosulfan by mixed bacterial culture could be due to the synergistic effect of bacterial isolates or the mixed bacterial culture might contain predominantly certain class of bacteria that have wide range of substrate specificity.

F. Pesticide Biodegradation in Soil Slurry

All the bacterial treatments were significantly effective in pesticide remediation over control in soil slurry. Residual level of pesticides in different treatments is depicted in Table IV. Pattern of carbendazim degradation was, consortium (88.4%) > GB61 (86%) > GB21 (83.6%) > GB20 (78.6%) > control (18.2%). Residual level of carbendazim was 11.6-81.7% after 20 days with various treatments. Significant degradation of endosulfan was observed in all the treatments as compared to control. Highest degradation rate of α- and β- endosulfan was observed in consortium (87 and 83%) respectively as compared to control which showed only 27 and 24% degradation respectively. As compared to control there was ≈3 fold increase in degradation rate of endosulfan by the consortium. A significant effect of bacterial treatment over control was also observed on imidacloprid. An increase of 3.6 fold in degradation rate of imidacloprid was observed in consortium as compared to control. Kumar [30] have also studied degradation of endosulfan in soil slurry and observed 63-75% degradation in 10 days by different soil isolates. They reported 73 and 55% degradation within 10 days by mixed cultures. A similar study was conducted by Murthy et al. [31] on biodegradation of hexachlorocyclohexane in soil slurry in a lab scale bioreactor by a defined bacterial consortium under aerobic conditions. Ten and 25 ppm of HCH were degraded completely by 120 and 168 h respectively. At both the concentrations, γ -isomer of HCH was degraded faster than β. Irvine et al. [32] demonstrated the successful use of a soil slurry batch reactor for biotreatment of soils contaminated with petroleum hydrocarbons. They observed Total Petroleum Hydrocarbon (TPH) removal efficiencies were greater than 96% in slurry reactors supplemented with nutrients.

G.FT-IR Analysis

FTIR studies were conducted to identify the presence of bonding or stretching vibrations in the biodegraded pesticides. FTIR spectrum of the control (carbendazim) compared to it's biodegraded metabolites is shown in Fig. 1. In the standard, peak at 1619.91 cm^{-1} may be assigned to C=N stretching which is observed to produce bands between 1200 and 1310cm^{-1}. The peaks below 1200 cm^{-1} may be attributed to C-H bending vibrations of aromatic and heterocyclic rings [33]. FTIR spectrum of consortium degraded carbendazim showed the appearance of a very broad peak at 3500 (for O-H stretching). The shift in peak pattern and occurrence of few new peaks attributes to biodegradation of carbendazim. IR spectra of endosulfan shows characteristic absorptions at 1600, 1270, 1192 and 750 cm^{-1} which attributes to the presence of C=C, C-O, S-O and C-Cl bondings [34]. Consortium showed characteristic absorption particularly at 3400 and 1270 cm^{-1} (O-H and C–O stretching) and absence of absorption at 1192 cm^{-1} (S-O) which indicates the presence

endosulfan alcohol**.** FTIR spectrum of control (imidacloprid) displays peaks at 1570 cm^{-1} for the vibration band of N=N in the imidazolidine, 1520, 1450, 1430 for –CH= aromatic rings and 1350 cm^{-1} for NO_2 stretching of the compound. FTIR spectrum of biodegraded imidacloprid showed the appearance of a few new peaks compared to control. Main peaks were present at 3400-3150 (O-H stretching of R-COOH), bonded OH and at 1680 cm-1 for C=O stretching of metabolites. Peak pattern of consortium was totally different from rest of the isolates showing the effectiveness of consortium in biodegradation.

H.GC-MS of Biodegraded Products of Endosulfan

Samples of biodegraded endosulfan in soil slurry were analysed by GC-MS (Table V). The mass spectrum of biodegraded endosulfan by GB69, showed production of two compounds, Benzene, (1-ethylnonyl) and Propenal, 3(2, 6, 6-trimethyl-1-cyclohexen-1-yl) at retention time of 18.6 and 21.653. Isopropyl laurate, 2-3-methyl-2-(2-oxopropyl) furan and Propenal, 3 (2, 6, 6-trimethyl-1-cyclohexen-1-yl) were detected at 13.832, 14.764 and 21.653 minutes respectively in GBA treatment. Phenyl glycine (MW=151), 3-methyl-2-(2-oxopropyl) furan (MW=138) and Pyrene (MW=202) were detected in GB72. In consortium, (3E)-2,2,3,4,5,5-hexamethylhex-3-ene, isotoralactone, cyclobutanone,2-tetradecyl and 3(2,6,6-trimethyl-1-cyclohexen-1-yl) were detected at a retention time of 14.79, 19.079, 20.085 and 21.653 min. respectively. The results showed that the numbers of intermediates formed during biodegradation of endosulfan by mixed-bacterial culture were relatively more in number than the mono-culture, but no toxic intermediates (endosulfan sulphate, endosulfan monoaldehyde and endosulfan diol) were observed. The results suggested that the biodegradation of endosulfan did not produce any toxic intermediates, and thus could be utilized for the bioremediation process of endosulfan contaminated soil and water. Weir et al. [35] conducted the endosulfan degradation experiments with white rot fungi and a bacterial isolate. They observed that endosulfan was degraded via oxidation and hydrolysis and produced endosulfan sulfate (toxic) and endosulfan monoaldehyde, (less toxic) respectively. But, in the present investigation, none of the reported intermediates of endosulfan were formed nor accumulated in the system. Samples collected from endosulfan degradation experiments using bacterial isolates did not show any peak corresponding to endosulfan diol and endosulfan ether in GC/ECD, but some other compounds were observed in GC-MS.

I. In situ Biodegradation of Pesticides in a Pot Experiment on Onion

All the bacterial treatments performed significantly better in relation to plant dry weight and plant height over control (Table VI). Maximum plant height was observed in GB20 (28.33cm) treated plants under the influence of carbendazim, which was 1.17 fold higher than control (Table VI). Highest plant height (endosulfan and imidacloprid treatment) was observed in GBA (28.23 cm) and GB35 (27.33cm). In carbendazim, other treatments were ineffective for plant dry weight except GB20 and GB21. Maximum plant dry weight was observed in GB20 with an increase of 1.12 fold over control. Similarly in imidacloprid treated soil, plant dry weight was more in GB5 and GB35 as compared to control. Rhizospheric degradation results indicated a significant effect of bacterial treatment over control. A lower degradation rate was observed at higher dose (100mg/kg) in comparison to 50 mg/kg in all the pesticides. In bacterial treatments, onion plants showed lesser uptake of pesticides. The concentration of carbendazim in the rhizosphere soil (spiked with 50mg/kg and 100mg/kg carbendazim) at the end of 30 days was minimum in consortium (1.33mg and 3.33mg) respectively. Least uptake of carbendazim by onion plants was recorded in GB21 (11.97µg and 75.27 µg) at 50mg/kg and 100mg/kg respectively.Maximum removal of carbendazim was observed in consortium (94% and 92%) in the soil spiked with 50mg/kg and 100mg/kg carbendazim respectively. Percent carbendazim removal in bacterial treatments was in range of 85-94%. Residual level of endosulfan in onion plants was low in the bacterial treatments as compared to control. Minimum level of endosulfan residue was detected in GB72 treated plants, followed by GBA**.** Minimum residual level of α-endosulfan (2.43 and 7.23 mg) and β-endosulfan (3.10 and 8.83 mg) in soil spiked with 50 and 100mg/kg endosulfan respectively was observed in consortium (Table VI). Maximum removal of both the isomers was observed by consortium, followed by GBA. All the bacterial treatments performed significantly better than the control in imidacloprid degradation (Table VI). In control, imidacloprid residue in plant parts were 47.67 and 122.00 µg/g in soil spiked with 50 and 100mg/kg imidacloprid respectively, whereas the level of imidacloprid was 21.20-22.90 µg/g and 86.37-95.77 µg/g in presence of bacterial treatments under 50 and 100 mg/kg pestcide. Self degradation of imidacloprid was 16.74 and 10.44% in soil under same concentration. Bacterial isolates enhanced removal of imidacloprid maximally (79.9%) within 30 days**.** Maximum degradation of imidacloprid was recorded in consortium treatment with residual imidacloprid of 4.5 and 9.43 mg in soil spiked with 50 and 100mg/kg imidacloprid respectively. Findings of this study are in accordance with Dubey and Fulekar [36] who studied rhizoremediation of cypermethrin using a pot culture experiment in *Pennisetum pedicellatum* rhizosphere. Bioremediation data of cypermethrin by strain *Stenotrophomonas maltophilia* MHF ENV 22 examined by HPLC and mass spectroscopy, indicated 100, 50 and 58% degradation within 72, 24 and 192 h at 25, 50 and 100 mg/kg, respectively. They have also reported lower degradation percentage at higher doses. However in a similar study conducted on lindane degradation in spinach rhizosphere by Pandey et al. [12] reported increased degradation of lindane at higher doses. Benimeli et al. [37] conducted a study on lindane bioremediation abilities of *Streptomyces* sp. M7 and its effect on *Zea mays* using different concentrations of lindane in soil. Benimeli et al. [37] reported 68% degradation of 100 µg/kg of lindane in non-sterile soil after 14 days by *Streptomyces* sp. but did not find any involvement of

indigenous microflora in bioremediation. A similar study on lindane remediation under maize and rice rhizosphere conducted by Phartyal, [38] who reported upto ≈88-94% and ≈76.5-78% degradation of lindane by *Bacillus subtilis* under maize and rice rhizosphere respectively.

TABLE I
RESIDUAL LEVEL OF PESTICIDES IN SOIL OF AGRICULTURE FIELDS

Place	Pesticide Used	Residual Level Of Pesticides In Soil (µg/g)
Bajpur	Imidacloprid	0.02133
	Carbendazim	0.0306
	Chlorpyriphos	0.5773
	Endosulfan	0.0173
Lalkuan	Imdacloprid	0.0392
	Carbendazim	BDL
	Chlorpyriphos	0.7176
	Endosulfan	BDL
Haldwani	Imdacloprid	0.1666
	Carbendazim	BDL
	Chlorpyriphos	0.7504
	Endosulfan	BDL

[a]Pesticide residue **(**ug/g**)** below detected level

TABLE II
TOLERANCE LEVEL OF BACTERIAL ISOLATES FOR DIFFERENT PESTICIDES

Bacterial Isolates	Maximum Tolerance Level (µg/ml)		
	Carbendazim	Endosulfan	Imidacloprid
GB20	150	-[a]	-
GB21	150	-	-
GB61	130	-	-
GB69	-	170	-
GB72	-	150	-
GBA	-	130	-
GB5	-	-	110
GB78	-	-	150
GB35	-	-	150

[a] (-) not determined

TABLE III
PHYLOGENETIC POSITION OF SELECTED BACTERIAL ISOLATES

Isolates	Closest BLAST[a] match	Isolates Identified
GB20	*Bacillus pumilus* strain BFB30	*Bacillus sp* GB20
GB21	*Exiguobacterium profundum*	*Exiguobacterium* GB21
GB61	*Achromobacter* sp. strain R-2079	*Achromobacter* GB61
GB69	*Xanthomonas* sp. PHLE-1	*Xanthomonas* GB69
GB72	*Microbacterium* sp. B-2013	*Microbacterium* GB 72
GBA	*Bacillus aryabhatti* strain SCSAAB0010	*Bacillus* sp. GBA
GB5	*Achromobacter* sp. strain R-46660	*Achromobacter* GB5
GB35	*Pseudomonas sp.*HY8N	*Pseudomonas* GB35
GB78	*Microbacterium* sp. B-2013	*Microbacterium* GB78

[a]basic local alignment search tool

TABLE IV
IN VITRO BIODEGRADATION STUDIES OF PESTICIDES IN BROTH, SOIL AND SOIL SLURRY AFTER 20 DAYS

Bacterial isolates	% Degradation of Carbendazim		
	Minimal broth	Soil	Soil slurry
Control	16.4	17.2	18.2
GB20	73.4	76	78.6
GB21	77.7	81	83.6
GB61	81.98	83.2	86
Con[a]	83.73	84.4	88.4
	% Degradation of Imidacloprid		
Control	18	17.1	21
GB5	62	63	67
GB78	60	65	72
GB35	64	62	68
Con	82	69	75. 7

	% degradation of α and β isomers of Endosulfan					
Control	22	20	24.2	20	28	25
GB69	82	80	80	76.3	82.5	78
GB72	84	78	82.6	78.3	87	83
GBA	80	78	79.2	74	80	80
Con	81	82	84.6	81.3	87	83

[a]con= consortium of three bacteria

TABLE V
BIODEGRADED METABOLITES OF ENDOSULFAN USING GC-MS

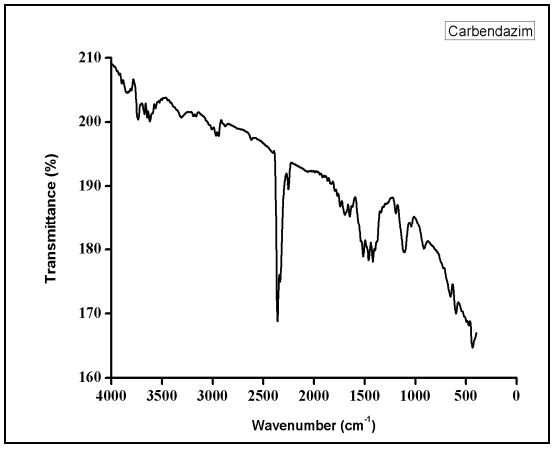
Bacterial isolates	Intermediate compound	R.T
GB69	Benzene	18.6
	Propenol	21.653
	Isopropyl laurate	13.832
GBA	2-3 methyl 2-(2- oxopropyl) furan	14.79
	Propenal	21.65
	Phenylglycine	16.913
GB72	3-methyl-2-(2-oxopropyl) furan	13.85
	Pyrene	14.79
	(3E)-2,2,3,4,5,5-hexamethylhex-3-ene	14.79
Consortium	isotoralactone	19.079
	cyclobutanone	20.085
	2-tetradecyl	21.653

[a]RT= Retention time of compound for GC-MS

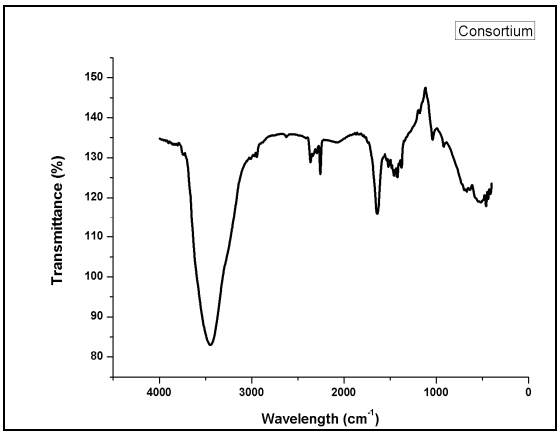
TABLE VI
PLANT PARAMETERS AND RESIDUE ANALYSIS OF CARBENDAZIM [A]

Treatments	Plant height (cm)		Plant dry weight (g)		Pesticide uptake by plant (µg)		Pesticide Residue in soil (mg)	
	50mg/kg	100mg/kg	50mg/kg	100mg/kg	50mg/kg	100mg/kg	50mg/kg	100mg/kg
C1	NA	NA	NA	NA	NA	NA	19.77	39.96
C2	24.1	25.60	5.53	6.03	34.1	107	18.53	38.6
GB20	28.3	27.4	6.23	5.47	14.0	82.77	2.7	6.53
GB21	24.8	26.87	6.2	5.03	11.9	75.27	2.63	5.60
GB61	27.2	28	5.1	4.87	12.9	78.17	2.23	4.77
CON	25.6	25.23	4.9	5.33	13.2	84.00	1.33	3.33

[a]cm= centimeter, g=gram, µg=microgram, mg=milligram, kg=kilogram, NA= not analyzed, CON= consortium, C1&C2 = control



(a)



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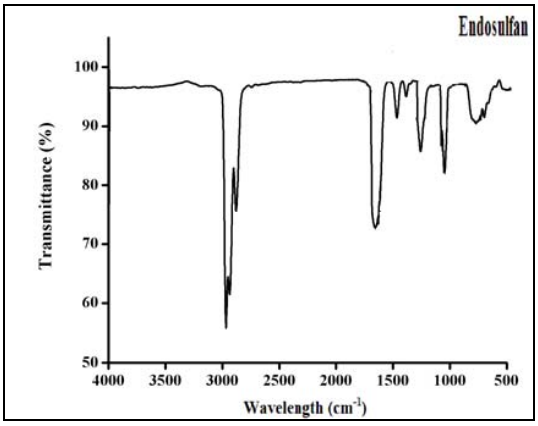

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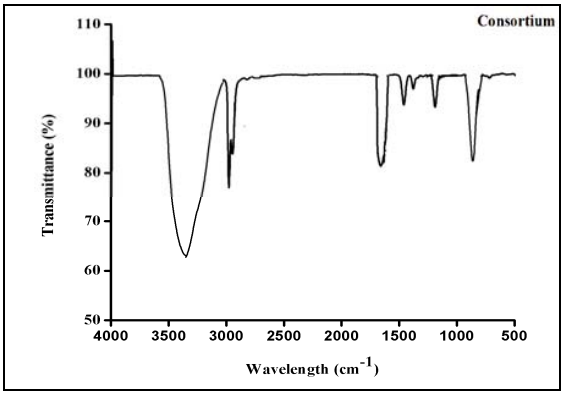

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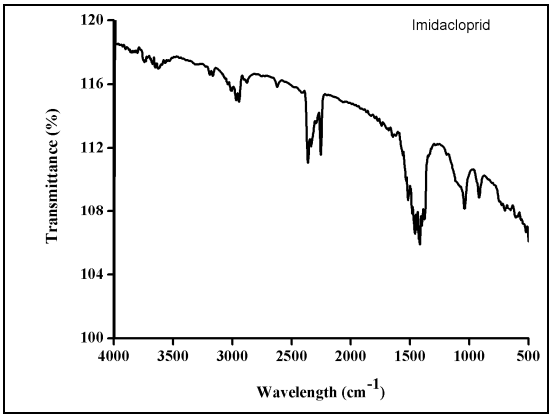

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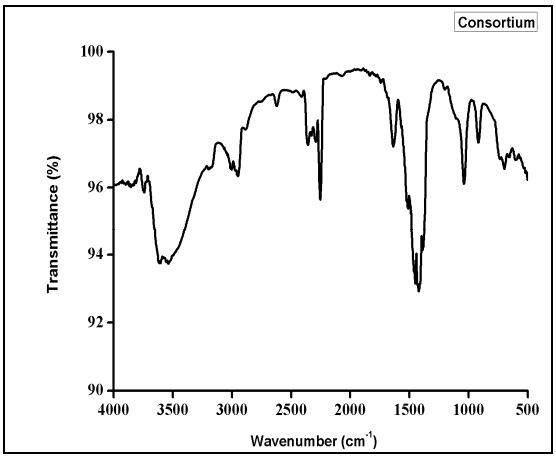

(f)

Fig. 1 (a)-(f) FT IR of Carbendazim, Endosulfan and Imidacloprid degraded by bacterial consortia after degradation

TABLE VII
PLANT PARAMETERS WITH ENDOSULFAN[A]

Treatments	Plant height (cm)		Plant dry weight (g)	
	50mg/kg	100mg/kg	50mg/kg	100mg/kg
C1	NA	NA	NA	NA
C2	23.83	25.27	5.47	6.10
GB69	27.90	27.13	5.30	5.83
GB72	25.37	27.00	6.03	5.47
GBA	27.00	28.23	6.17	5.00
CON	25.87	25.60	5.57	5.57

[a]cm= centimeter, g=gram, mg=milligram, kg=kilogram, NA= not analyzed, CON= consortium, C1&C2 = control

TABLE VIII
ENDOSULFAN RESIDUE IN PLANT AND SOIL[A]

Treatments	Pesticide uptake by plant (µg)				Pesticide Residue in soil (mg)			
	α- endosulfan		β- endosulfan		α- endosulfan		β- endosulfan	
	50mg/kg	100mg/kg	50mg/kg	100mg/kg	50mg/kg	100mg/kg	50mg/kg	100mg /kg
C1	NA	NA	NA	NA	19.57	40.27	19.8	40.83
C2	40.83	117.67	42.67	122.3	18.63	38.33	19.2	40.33
GB69	17.67	91.40	19.33	93.17	2.9	8.87	3.87	9.97
GB72	15.33	84.57	17.33	85.60	2.8	8.43	3.7	10.33
GBA	15.97	82.27	18.00	88.57	2.43	8.73	3.17	9.9
CON	16.87	86.00	18.00	89.07	2.43	7.23	3.10	8.83

[a] µg=microgram, mg=milligram, kg=kilogram, NA= not analyzed, CON= consortium, C1&C2 = control

TABLE IX
PLANT PARAMETERS AND RESIDUE ANALYSIS OF IMIDACLOPRID[A]

Treatments	Plant height (cm)		Plant dry weight (g)		Pesticide uptake by plant (µg)		Pesticide Residue in soil (mg)	
	50mg/kg	100mg/kg	50mg/kg	100mg/kg	50mg/kg	100mg/kg	50mg/kg	100mg/kg
C1	NA	NA	NA	NA	NA	NA	18.73	40.3
C2	24.30	26.10	5.57	5.90	47.67	122.00	17.67	39.03
GB5	24.50	24.37	6.00	5.83	22.90	95.77	5.23	11.4
GB35	27.33	28.33	6.13	5.50	21.20	92.03	5.27	11.9
GB78	26.20	26.53	5.83	5.14	21.80	86.37	4.97	11.3
CON	26.60	25.67	5.93	5.37	22.20	93.37	4.5	9.43

[a]µg=microgram, mg=milligram, kg=kilogram, NA= not analyzed, CON= consortium, cm=centimeter

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