Development of Fungal Consortium to Degrade the Polyethylene Polymer and Polystyrene

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Abstract: - In our day-to-day life we are facing many problems and struggles. In that, the one of the main problem is plastic accumulation. Plastic is a synthetic polymer that is widely used in almost every field of life. The massive use of this synthetic polymer has led to the accumulation of this polymer in the environment thus polluting the environment. The general techniques in preventing plastic waste as landfill, incineration, recycling are considered less effective as they release some hazardous materials to the environment. Since last few decades the uncontrolled use of plastics for various purposes such as packaging, transportation, industry and agriculture in rural as well as urban areas, has elevated serious issue of plastic waste disposal and its pollution. The efficient decomposition of plastic bags takes about 1000 years. Thus, the appropriate technique is needed to overcome this problem. Biodegradation is an enzymatic degradation involving some microorganisms including bacteria, fungus. The work presented here for the degradation of polyethylene polymer and polystyrene by the fungal isolate. Seven soil samples were collected from different sites in and around Coimbatore and Tirupur district. Isolation was done by the serial dilution and spread plate method in that we took *Aspergillus niger, Aspergillus flavus, Fusarium* sp, *Penicillium* sp. Fungi were isolated on Potato Dextrose Agar (PDA) medium and screen on minimal salt medium (MSM) and Potato Dextrose Broth supplemented with polyethylene powder and polystyrene. Four fungi inoculated together in a Potato Dextrose Broth supplemented with polystyrene. The extent of biodegradation on the polyethylene polymer and polystyrene was assessed by Fourier Transform Infrared Spectroscopy (FTIR) techniques. This technique can be used to prevent the plastic waste problem.

Key Words: —*Plastic degradation, Fungi, Polyethylene Polymer and Polystyrene and FTIR*.

I. INTRODUCTION

The word plastic comes from the Greek word plastikos, which suggests ‘able to be molded into varied shapes. Plastic is defined because the polymer, which become mobile on heating and thus may be cast into moulds. The plastic is created of carbon, hydrogen, silicon, oxygen, chloride and nitrogen. For extraction of the fundamental materials of plastics oil, coal and gas are used (Swapnil et al., 2015). Mostly used plastics are polyethylene (LDPE, MDPE, HDPE and LLDPE), Poly Ethylene Terephthalate (PET), Polybutylene Terephthalate (PBT), nylons, Poly-Propylene (PP), Polystyrene (PS), vinyl resin (PVC), and Polyurethane (PUR) (Himani et al.,2012).

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Polyethylene is one in all the foremost valuable synthetic non-biodegradable polymers made from elements that are a thermoplastic polymer made up of repeating units of ethylene. Degradation of polyethylene is of great challenge because the materials are increasingly used. Microorganisms biologically transform organic complex polymer to simpler one and utilized it as a carbon source. The microorganisms secrete several enzymes in numerous quantities, which expressed its degradation efficiency of the microorganism. Polyethylene contains the chemical elements carbon and hydrogen. In terms of degrading ability of polyester polyurethanes variety of microorganisms, principally fungi are characterized. Microorganisms play a big role within the decomposition of fabric. In most studies, fungi are investigated for the biodegradation of PE because these organisms produce degrading enzymes and, extracellular polymers (such as polysaccharides) facilitate to colonise the polymer surface, and also the ability of distribution and penetration of the fungal hyphae is a plus (Anchal et al.,2015).

A. Plastic Waste In India

As much as 3.3 million metric tonnes of plastic waste was generated in India in 2018-19, consistent with the Central Pollution control panel (CPCB) report 2018-19. This roughly translated to 9,200 tonnes every day (TPD).The overall municipal solid waste generation is 55-65 million tonnes; plastic waste is approximately 5-6 per cent of the overall solid waste generated within the country. Goa has the very best per capita plastic waste generation at 60 grams per capita per day, which is almost double of what Delhi generates (37 grams per capita per day).

The annual report was compiled supported submissions from the state pollution control boards (SPCB), though the source of the information provided is unclear, as no state-wise survey has been conducted to date.

It's important, however, to notice that the estimations of the report are substantially not up to those mentioned within the 2015 CPCB report on Assessment and quantification of Plastic Waste Generation in major Cities. It extrapolated data supported the findings from 60 cities in India. It reported that near 25,940 TPD (approximately 9.4 million tonnes per annum) of plastic waste was generated within the country .Clearly, we don't know the quantity of plastic we generate as a rustic, because the increase in wealth and affluence contributes to a better generation of plastic waste.

Despite the Plastic Waste Management legislation of 2011, followed by numerous changes within the recent past, most parts of the country lack systematic efforts required to mitigate the risks related to plastic waste.

The states started providing data on the identical only in 2018-19 for the primary time. A legal obligation has been reduced to a mere formality, and there is an absence of concern, motivation, awareness, compliance and enforcement of the foundations.

B. Draft Plastic Waste Management Rules, 2021

The draft Plastic Waste Management Rules, 2021, issued by the Union Ministry of Environment, Forests and global climate change (MoEFCC) on March 11, has necessitated some changes within the country's handling of its plastic waste. One, the amendment has extended the applicability of the principles to brand-owner, plastic waste processor, including their cycler, co-processor, etc. It will also include new definitions of: Non-woven bag, Plastic Waste processing, Single-use plastic(SUP)item, Thermoset plastic, Thermoplastic The Union ministry has proposed increasing the thickness of carry bags fabricated from virgin plastic to 120 microns from 50 microns. The draft also proposes a ban on the manufacture, import, stocking, distribution, sale and use of specific single-use plastic from January 1, 2022. These include plastic sticks for balloons, plastic flags, candy sticks, ice-cream sticks, and thermocol (extended polystyrene) for adornment. The draft is open for public suggestion for 60 days for consideration by the central government, following which it will be published within the Gazette of India. These rules could also be called Plastic Waste Management (Amendment) Rules, 2021, and shall inherit force on the date of publication within the Official Gazette.

C. Plastics Hazardous Substances

Plastics contain some hazardous substances affecting human health. Dangerous plastic components like bisphenol A found in PC and PVC can cause the system disorders, mainly the ovaries. Phthalates contained in PS and PVC result in testosterone disorders and interfere with sperm motility. The styrene monomers as those found in polystyrenetype plastics are carcinogenic. Nonylphenol contained in PVC causes estrogen disorders. Meanwhile, dioxins, persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) found in the majority plastic types leading to various health problems. Dioxins are carcinogens interfering with testosterone disorders. POPs can disrupt the nervous and reproductive systems. PAHs related to the genital system and development disorders, and PCBs associated with internal secretion disorders (Angga Puja Asiandu et al., 2021).

D. Description Of Problem

Plastic debris in the environment poses a significant threat because of its resistivity to photooxidative, thermal, mechanical and biological processes. Although overlooked for many years, the amount of plastic debris accumulating in the environment has been steadily increasing because of the material's durability and lightweight nature. Once discarded on land plastic debris makes its way to water bodies that act as sinks for low- density litter. Topography, wind and water currents, and proximity to pollution sources control the amount and types of plastics along shorelines, whereas degradation processes determine how long plastic debris remains on beaches an estimated 300 million tons of plastics are produced yearly. Plastics are human- made materials manufactured from polymers or long chain of repeating molecules. They are determined from oil, natural gas and increasingly from plant like corn and sugarcane. About 4%

of the world's petroleum is used to make plastic, and another 4% is used to power plastic manufacturing processes. Polyethylene (PE) represent about a third of total plastic production, with PE is largely utilized in packaging. Plastic debris, an inevitable consequence of living the 'Plastic Age', is dominating our lakes and oceans and poses a worldwide threat to aquatic wildlife.

II. Materials And Method

A. Collection of Sample

Seven different types of soil samples were collected from in and around Coimbatore and Tirupur district. The seven soil sample (A, B, C, D, E, F, G) were indigenous to locations:(A) Soil from Poultry Farm – 1,(B) Soil from Poultry Farm – 2,(C) Soil from Chicken Cage,(D) Fertile soil,(E) Chicken feaces, (F) Soil from Dump site (Waste),(G)Soil from Agricultural Land. The soil samples were collected at a depth of 3-5cm, in a sterile container. The samples were sealed properly, labelled and transported to the laboratory. All the samples were processed within 24 hours of collection.

B. Isolation of Fungi

The samples were subjected to serial dilution to urge diluted and inoculated on to the sterile petridishes, which contain Potato Dextrose Agar. The serial dilution spread plate method was followed to isolate fungi from soil samples. 1g of the effluent soil samples was added to 100 ml of H_2O and was serially diluted upto 10^{-7}. The fungi was isolated by spread plating 0.1 ml of every of the dilution on potato dextrose agar plates. The plates were kept for incubation at room temperature for 4 days to get colonies. The individual colonies were picked upon the idea of their characters like shape, surface, appearance and colour. They were subcultured again to potato Dextrose Agar and Rose Bengal Agar. Fungus was identified on the idea of colony characteristic (macroscopic), microscopic view Lacto-phenol cotton blue was wont to stain the fungi for microscopic view and for the spore's arrangement.

C. Preparation of Polyethylene Powder

Polyethylene sheets were cut in to small bits and immersed in xylene and boiled for 15 min. xylene dissolve the LDPE and also the residue was crushed while it absolutely was warm by hand with help of pestle and mortar. The LDPE powder so obtained was washed with ethanol to get rid of residual xylene and allowed to evaporate (3 h) to get rid of ethanol. The powder was dried room temperature. The LDPE powder was stored in closed containers in room temperature.(Plate1)

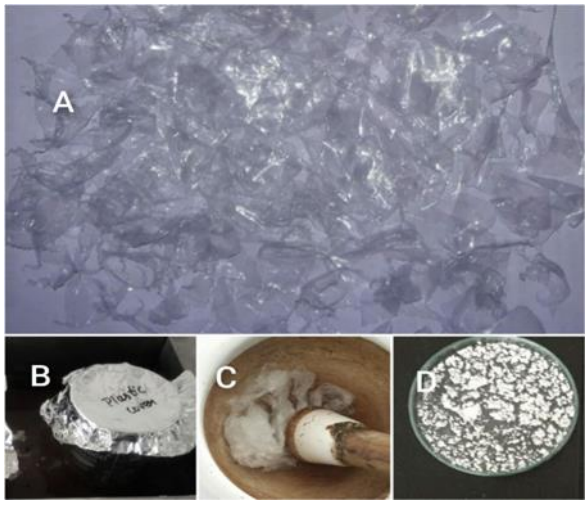

Fig.1. PLATE: 1 A – Polyethylene Cover Pieces, B – Plastics Immersed In Xylene Being Processed In Water Bath, C – Residue Was Crushed In Pestle And Mortar, D – Xylene Evaporation.

D. Preparation of Polystyrene Powder

Polystyrene immersed in xylene and boiled for 15 min. xylene dissolve the LDPE and the residue was crushed while it had been warm by hand with help of pestle and mortar. The LDPE powder so obtained was washed with ethanol to be rid of residual xylene and allowed to evaporate (3 h) to get rid of ethanol. The powder was dried room temperature. The LDPE powder was stored in closed containers in room temperature.(Plate 2)

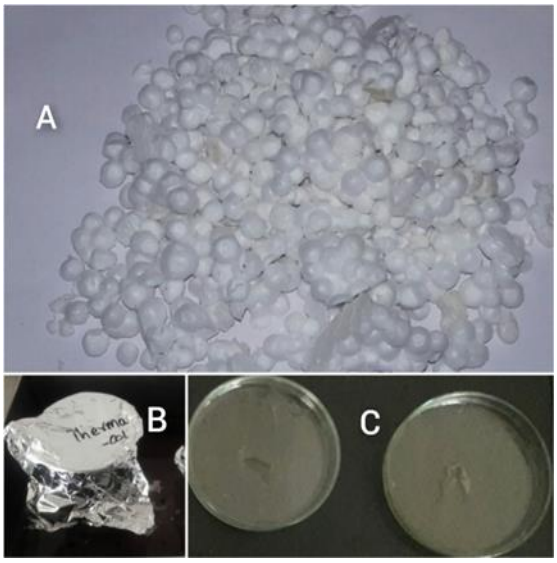

Fig.2. PLATE: 2 A – Polystyrene Pieces, B– Plastics Immersed In Xylene Being Processed In Water Bath, C – Xylene Evaporation

E. Evaluation of Polyethylene Degradation

Polyethylene powder was added in minimal salt medium (MSM) containing (g/l of distilled water): NH4NO3, 1.0; MgSO4.7H2O, 0.2; K2HPO4, 1.0; CaCl2.2H2O, 0.1; KCl, 0.15; and yeast extract, 0.1; and 1.0 mg/l of every of the subsequent micro-elements. FeSO4.6H2O, ZnSO4.7H2O and MnSO4;Polyethylene powder was added in minimal salt medium and potato dextrose agar at a final concentration of 0.1% (w/v) respectively and therefore the mixture was sonicated for 1 hour at 120 rpm in shaker. After sonication, the medium was sterilized at 121°C and pressure for 15 lbs for quarter-hour. About 15 ml sterilized medium was poured before cooling in each plate All morphologically distinct colonies were selected and streaked on minimal salt medium containing LDPE powder as a sole carbon source. The fungi were allowed to grow at 30-35 ^{0}C for 1 month 25 days.

F. Evaluation of Polystyrene Degradation

Polystyrene was added in potato dextrose broth at a final concentration of 1g respectively and the mixture was sonicated for 1 hour at 120 rpm in shaker. After sonication, the medium was sterilized at 121°C and pressure for 15 lbs for quarter-hour. All morphologically distinct colonies were selected and streaked on PD Broth containing Polystyrene as a sole carbon source. The fungi were allowed to grow at 30-35 ^{0}C for 1 month 23 days.

G. Development of Fungal Consortium on Liquid Medium

Prepare 600ml of PD broth in 1000ml conical flask. Then the medium was sterilized at 121°C and pressure for 15 lbs for 15 minutes. After sterilization, inoculate the PE degrading fungi isolate from different soil sample. Add 5g of polystyrene to PD broth and incubate at room temperature for 39 days.

H. Qualitative Analysis of Polystyrene Degradation

Polystyrene degradation was confirmed using Fourier transform infrared (FTIR) spectroscopy

I. FTIR Analysis

Fourier Transform infrared (FTIR) Spectroscopy were carried out to analyze the degradation of polystyrene with fungal cultures. Shifting in the position of peaks after fungal treatment was observed by FTIR analysis. FTIR results were analyzed using standard chart available. FTIR spectra were carefully examined, changes were observed in various peaks at different wavelength ranges that also correspond to degradation of polystyrene. The sample was characterized by transform infrared spectroscopy (FTIR) spectra measurement in the frequency range of 4,000–500 cm^{-1}.

III. RESULTS AND DISCUSSION

A. Soil Sample Collection

Totally seven soil samples were collected from different location of in and around Coimbatore and Tirupur districts was mentioned in Plate 3.

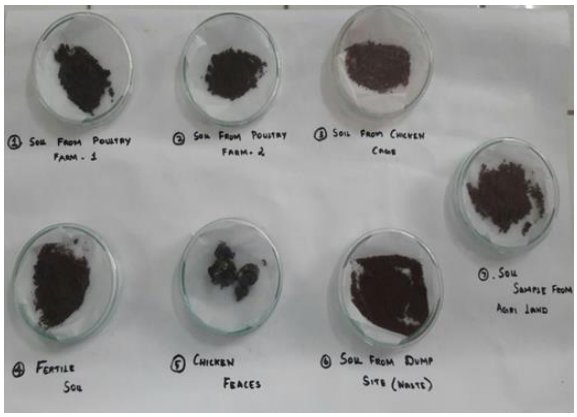

Fig.3. Plate: Three Soil sample collected from various region

B. Isolation and Identification of Fungi

Four different fungal species were isolated.The fungal strains were identified by Macroscopic and Microscopic observation. The soil sample like poultry farm- 2 (2) , Fertile soil (4) , Chicken feaces (5) , Soil from dump site (Waste) (6) were used to isolate the fungi *Aspergillus niger* , *Aspergillus flavus* , *Penicillium* sp , *Fusarium* sp .

The first fungal species exhibited wooly appearance and produced white spores that turned into black spores after a while. The LPCB staining of this species showed long, smooth conidiophores and biseriate phialides that covered the entire vesicle and forms “radiate” head. Thus the organism was identified as ***Aspergillus Niger***.(Plate 4)

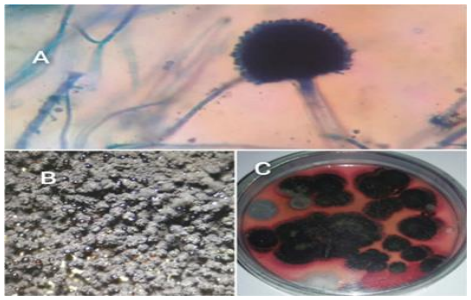

Fig.4. Plate: 4 A – LPCB Image, B - Stereomicroscopic Image , C – Aspergillus niger In RBM Plate

The second fungal species exhibited velvety colony and yellow to green colour spores. The LPCB staining of this species showed rough, spiny conidiophores, uniseriate and biseriate phialides that covered the entire vesicle and pointed out in all directions. Thus the organism was identified as ***Aspergillus flavus.***(Plate 5)

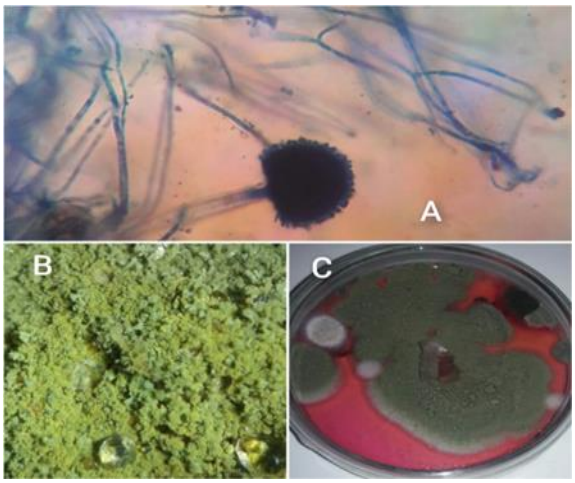

Fig.5. Plate: five A – LPCB Image, B - Stereomicroscopic Image, C – *Aspergillus flavus* In RBM Plate

The third fungal species exhibited woolly, and cottony textured colony which is initially white and become blue green. The LPCB staining of this species showed "squashed flower- like" conidiophores with branched, flask shaped phialides. Thus the organism was identified as ***Penicillium* sp.**(Plate 6)

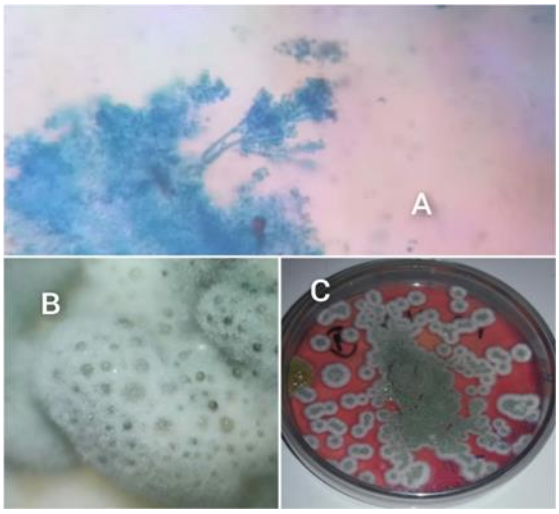

Fig.6. Plate: 6 A – LPCB Image, B - Stereomicroscopic Image, C – *Penicillium sp* In RBM Plate

The fourth fungal species exhibited bright white colored cottony aerial mycelium. The LPCB staining of this species showed micro and macroconidia from slender phialides. Thus the organism was identified as ***Fusarium* sp.**(Plate 7)

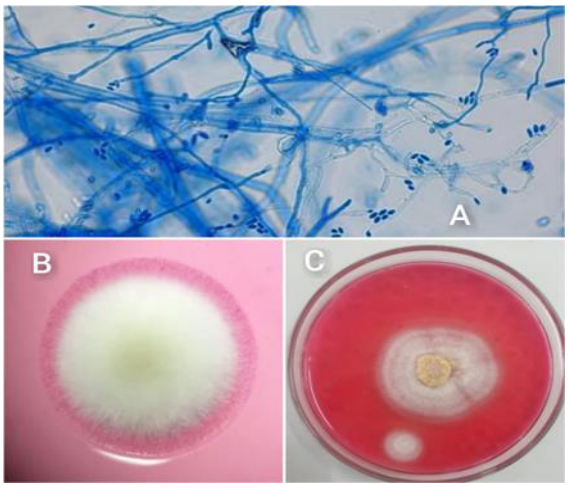

Fig.7. Plate: 7 A – LPCB Image, B - Stereomicroscopic Image, C – *Fusarium sp* In RBM Plate

C. Evaluation of Polyethylene Degradation

Minimal salt medium inoculated with different fungal species did not show any growth of microorganism and degradation of the polyethylene powder (Plate 8). The potato dextrose medium incorporated with polyethylene powder was inoculated with different fungal species. Among the other fungal species, ***Aspergillus Niger*** and ***Aspergillus flavus*** showed extraordinary degradation of polyethylene powder. The other fungal species showed less amount of degradation (Plate 9-12).

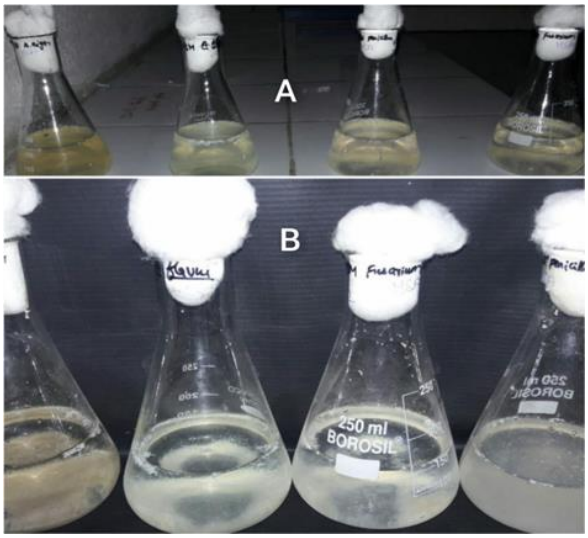

Fig.8. Plate: Eight-Polyethylene Powder In MSM Medium with Fungi: A- Before Degradation,

B – After 1 Month 25 Days Degradation,

D. Polyethylene Powder in PDA Plate with Fungi:

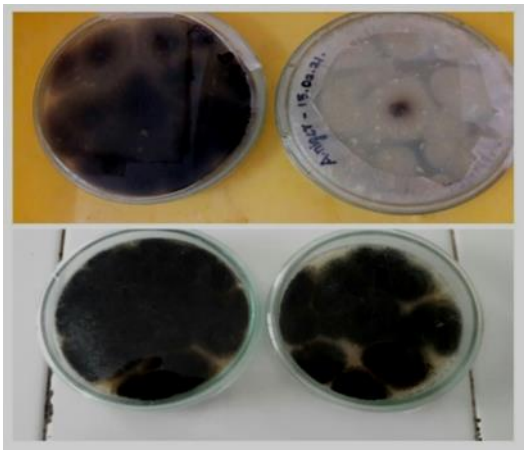

Fig.9. Plate: nine Aspergillus *niger*

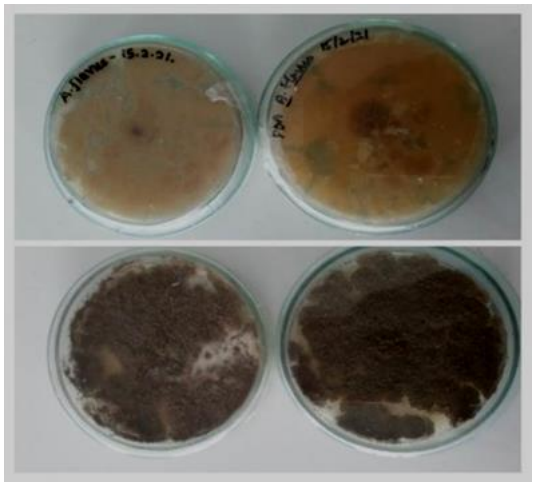

Fig.10. Plate: Ten *Aspergillus flavus*

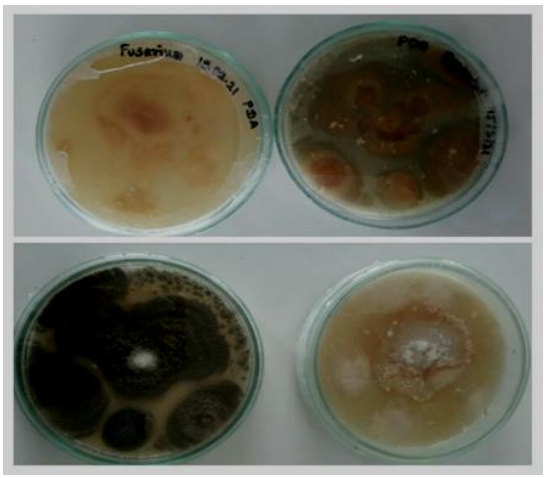

Fig.11. Plate: 11 Fusarium sp

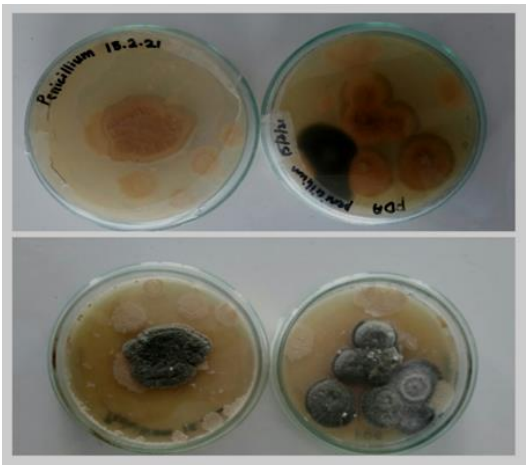

Fig.12. Plate: 12 *Penicillium* sp

E. Evaluation of Polystyrene Degradation

The fungi ***Penicillium* sp** showed **complete degradation** of the polystyrene pieces in the period of 53 days. The fungi ***Aspergillus Niger*** and ***Aspergillus flavus*** showed **partial degradation** of the polystyrene pieces in the period of 53 days. (Plate 13)

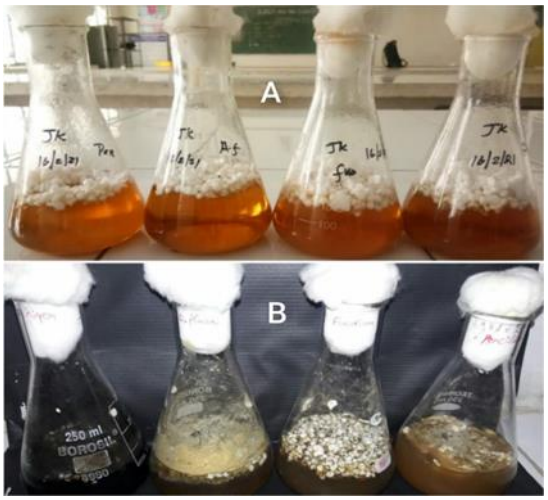

Fig.13. Plate: 13 Polystyrene Pieces in PDA Broth with Fungi, A – Before Egradation, B – After 1 Month 23 Days Degradation

F. Development of Fungal Consortium on Liquid Medium

In the fourth day, foam formation and gas production was seen. Degradation of the polystyrene pieces was seen gradually in the period of 39 days. After the particular period, the broth was analyzed using FTIR analysis for the qualitative analysis of the degradation of the polystyrene pieces. (Plate 14)

Fig.14. Plate: 14 A – Before Degradation, B – After 39 Days Degradation

G. Qualitative Analysis of Polystyrene Degradation

After FTIR Spectral analysis, (figure 1) bands are observed at 3647 $^{-1}$,3631^{-1} ,3626 $^{-1}$, 3508 $^{-1}$, 3518 $^{-1}$, which is O-H bond stretch free hydroxyl characteristic of alcohol and phenol, 1651-1 , 1645^{-1} that is characteristic of C=C stretch for alkenes, the absorption peak at 1506^{-1} is characteristic of N-O bond present in nitro compound.(Plate 15)

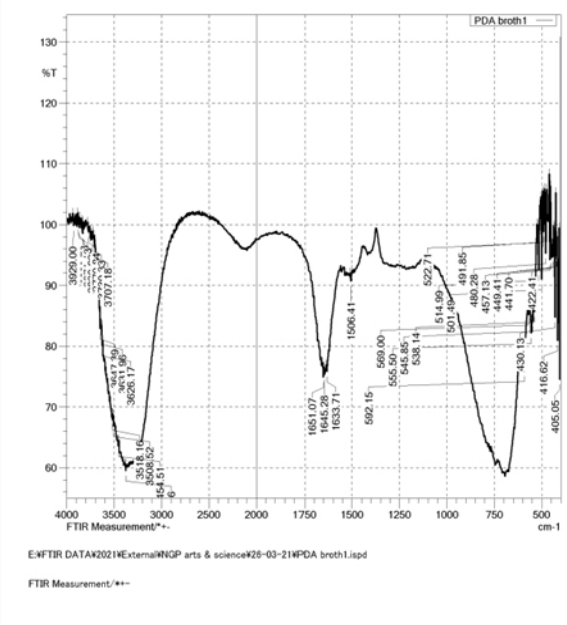

Fig.15. Plate: 15 FTIR Measurement

IV. DISCUSSION

Fungi were isolated from different types of soil from in and around Coimbatore and Tirupur district and to test their ability for polyethylene and polystyrene degradation in laboratory condition. From the soil sample identified four fungi are Aspergillus niger, Aspergillus flavus, Penicillium sp, and Fusarium sp, these organisms has the ability to degrade polyethylene and polystyrene. In MSM medium did not show any growth of microorganism and degradation of the polyethylene powder. The findings of present investigation showed that Penicillium sp showed complete degradation of the polystyrene pieces in the period of 53 days in PD broth.

Soil sample was collected from various sites. The samples were serially diluted and fungal species were isolated based on the growth on potato dextrose agar, rose Bengal agar and lacto phenol cotton blue staining. The sample to be degraded (polyethylene and polystyrene compounds) was converted into powder form using xylene. Minimal salt medium and potato dextrose medium was prepared and polyethylene and polystyrene compounds were added separately and observed for degradation. The fungi inoculated on the potato dextrose medium showed decent degradation of the sample. The fungi Aspergillus Niger and Aspergillus flavus showed excellent degradation. Potato dextrose broth was prepared and polyethylene and polystyrene compounds were added separately and observed for degradation. The fungi Penicillium sp showed complete degradation of the polystyrene pieces. Fungal consortium was prepared by adding all the fungal species to the potato dextrose broth incorporated with polystyrene pieces. After fourth day, foaming and gas production was seen. The broth showed partial degradation of polystyrene pieces in the 39th day and it was kept for further degradation. The degradation process was confirmed using FTIR analysis. The presence of the functional groups alcohols, alkenes and nitro compounds confirmed that enzyme production and reduction of the polystyrene compound occurred.

V. CONCLUSION

Plastic is one of the major pollutants that have no solution other than recycling. It takes years for a normal degradation of plastic compounds. My project aims to solve this problem. Microorganisms are known to produce a wide variety of enzymes and by using these enzymes complex molecules can be converted into simpler molecules. Microbial enzymes can be used for the degradation of plastic in a less period of time. In my research, Penicillium sp showed Complete degradation of the plastic in the period of 44 days.

VI. ACKNOWLEDGMENT

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VII. CONFLICTS OF INTEREST

The authors have no conflicts of interest to publish this research article in this journal.

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