Potential Implementations of Eco-enzyme

SANGEETHA, S.

(17PZO013)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In partial fulfillment of the requirements for the Degree of

MASTER OF SCIENCE IN ZOOLOGY

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Signature of the
Head of the department

22/4/19

Signature of
the Supervisor

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1. INTRODUCTION

Food is the third most important thing for living beings to live after air and water. This shows the importance of food for life. Food in general comprises fruits and vegetables as a major part which provides carbohydrates, proteins, fats, minerals and vitamins for the living body to sustain good health. In the developing world, enormous amount of fruit as well as vegetable solid waste are generated primarily due to higher production, lack of appropriate preservation and transportation process (Mahmood*et al*., 1998).

Fruits and vegetables are the most utilized commodities among all horticultural crops. They are consumed raw, minimally processed, as well as processed, due to their nutrients and health-promoting compounds. With the growing population and changing diet habits, the production and processing of horticultural crops, especially fruits and vegetables, have increased very significantly to fulfill the increasing demands. Significant losses and waste in the fresh and processing industries are becoming a serious nutritional, economical, and environmental problem. In United Nations Food and Agriculture Organization (FAO) has estimated that losses and waste in fruits and vegetables are the highest among all types of foods, and may reach up to 60%. The processing operations of fruits and vegetables produce significant wastes as by-products, and constitute about 25% to 30% of a whole commodity group.

The waste is composed mainly of seed, skin, rind, and pomace containing good sources of potentially valuable bioactive compounds, such as carotenoids, polyphenols, dietary fibers, vitamins, enzymes, and oils, among others. These phytochemicals can be utilized in different industries including the food industry, for the development of functional or enriched foods, the health industry for medicines and pharmaceuticals, and the textile industry for dyeing, among others. The use of waste for the production of various crucial bioactive components is an important step toward sustainable development. Also, these

types of organic waste results from peeling and trimming of fruits and vegetable in household kitchens and food industries (Bouallagui*et al.*, 2005).

The food based organic waste comprises a major fraction in increasing municipal solid organic waste due to urbanization and increasing standard of living all over the globe. The generation of food waste will increase up to 44% by 2025. Thus the management of organic solid waste (OSW) will grow into a major issue all over the globe.

Organic Solid Waste containing huge amount of organic matter which ultimately degrades to produce carbon dioxide and methane, as the conventional disposal of organic solid waste and results in serious environmental pollution and health risks problems to living organisms (Ariunbaatar*et al.*, 2014).

From an environmental perspective, there is a crucial need to develop appropriate alternate waste management technology for the utilization of organic wastes as well as to minimize the pollution problems created by them (Anto *et al.,* 2006, Neves *et al.*, 2008, Dhanalakshmi and Alwar, 2012).

The chemical complexity, easy degradability, higher moisture content and nutrient rich composition of organic food waste (Esra *et al.,* 2014), made them as a useful resource for the production of higher value added products such as fuels, chemicals and biochemical through fermentation process (Chanakya *et al.*, 1999, Sakai *et al.*, 2004, Wang *et al.*, 2005, Zhang *et al* 2013,Melikoglu*et al.,* 2013). Globally, the interest of biochemical products (organic acids, enzymes, biopolymers etc.) production from organic waste is increasing day by day (Bo *et al.,* 2016). Among them, the enzymes play an important role to achieve zero discharge of organic solid waste from different sector by improving biological remediation process to recover valuable resources. The enzymes which are presently used in environmental applications are quiet expensive because of the cost of production and purification. (Kavitha *et al.*, 2013).

Recently researchers are producing a mixture of crude hydrolytic enzymes throughfermentation process by which seem to be a good substitute and perform better than expensive single enzyme (Enu *et al.,* 2006 and Wei *et al.,* 2015). Many researchers suggested that if the crude enzymes activity of biological solution is higher, it can be used directly without any recovery process in a feasible and economical way (Parawira, 2012, Leung 2012, Kiran 2014).

One such valuable enzyme was developed by Dr. Rosukon from Thailand in 2006 through simple inexpensive method for various household and hospital cleaning methods and was named as garbage enzyme or eco-enzyme. Converting organic waste into eco enzyme is important for reducing the amount of organic waste piling up in landfills. A study by Sustainable Waste Indonesia found that as much as 60% of the total waste produced in Indonesia is organic waste (CNN Indonesia, 2018). Unfortunately, out of the total waste produced (organic and non-organic), only 7.5% are processed. The rest of the wastes are piled, burned, ignored and as much as 69% are transported to landfills. This is problematic because the capacities of landfills are limited.

Organic wastes that are piled up in landfill produces harmful gases to the environment and to the health of the people living around the landfill. At landfills, organic waste go through anaerobic decomposition process that produces methane, a greenhouse gas which has the capacity to trap heat 30 times more effective than carbon dioxide.

This harmful anaerobic process often occurs to organic wastes that are kept away from oxygen in-between non-organic waste (e.g. food waste wrapped inside plastic). Moreover, methane may also threaten the health of people who live around the landfill by replacing oxygen content in the air. Environmental and health issue that arise due to organic waste in landfills must be managed by reducing the amount of organic waste produced, as well as by processing the produced organic waste, such as through producing eco enzyme(Unakal *et al.,* 2012).

Eco-enzyme is an organic solution produced by the simple fermentation of fresh vegetable wastes, fruit wastes with addition of brown sugar and water. The fermentation creates natural chains of proteins, mineral salts, organic acids, alcohol and enzymes. The fermented solution has the capacity to breakdown, change, create and catalyze the functions that make it a wonderful cleaning agent in household as well as in industrial and medical applications.

Eco-enzyme can be utilized as a low-cost alternative to improve wastewater treatment processes by removing the impurities and bacteria (Khairul and Shamila 2012, Bhavani Prakash 2013, Nazim and Meera 2013). This enzyme is a complex organic substance of protein chains (enzyme), organic acids and mineral salts produced by fermentation of waste fruits, vegetables or its peels, sugar and water. The eco-enzyme functions similarly to enzymes in achieving a high degree of degradation within a shorter time. In garbage enzyme, sugar is used frequently as a substrate in fermentation processes, in the production of lactic acid, polyhydroxybutyrate, ethanol, pullan, xanthan gum and molasses has been widely used as a substrate in fermentation processes (Bae *et al.,* 2004).

The proponents of the eco-enzyme describes it as a complex organic substances of protein chains, mineral salts, juvenile hormones and also claim that it functions to decompose, transform as well as catalyze reactions. It is also claimed that the garbage enzyme functions differently in different concentrations (Arun *et al.,*2015).

The eco-enzyme shows strong synergistic activity towards harmful microbes.The disinfectant property inside an eco-enzyme is due to the alcohol and/or acetic acid content in this liquid. Alcohol (ethanol) and/or acetic acid are produced by the metabolic process of bacteria that are naturally present on fruit or vegetable scraps. Fermentation is undergone by the bacteria to obtain energy from carbohydrate in an anaerobic (without oxygen) condition and with alcohol or acetic acid (depending on the type of microorganism) as the byproduct. Yeast

and bacteria produce alcohol through fermentation, whereas most bacteria produce acetic acid. This fermentation process is a result of enzyme activities of the microorganisms and has a disinfectant property (Rahna *et al.,* 2011).

The production of garbage enzyme with higher activity of lipase, amylase andprotease is needed to cater for treatment of larger quantity of industrial waste activated sludge generated. This necessitates the optimizing of various parameters to improve the activity of crude enzyme mixtures to reduce their cost and cost of application (Saravanan *et al.,* 2012).

Despite the increase in production of fish feed, the lack of efficient, cheap and available feed on the market remains a serious problem in the farming of aquatic animals. Moreover, there are risks of contamination of these feeds if they are not well preserved.The need for good quality feed ingredients with improved nutritional value, economic viability and growing awareness of the environment has led to a rise in the use of exogenous enzymes in the diet of fish and shrimp in recent years. Enzymes are formed during growth of microorganisms (trophophase), as a result of oxidative metabolism and aerobic fermentation. This has been developed for people to make simple cleaning solution at home or in small scale business to ease global warming because minimizing the environmental pollution by utilizing the cabbages like kitchen waste, vegetable and fruit wastes from stalls and markets (Renge *et al.,* 2012).

Eco-enzyme is natural, safe, and good for the environment and use the magic of nature to produce extraordinary removal of dirt, oil,stain etc. resulted to customer satisfaction. Today, most enzymes are used to improve digestibility of phosphorus and carbohydrates from plant protein sources. In addition, some solutions composed only of enzymes are produced and applied in fish farming in particular in the treatment of water. This is the case of the eco-enzymes or garbage enzymes produced from fresh wastes of plants (Chowdhury,2014).

Eco-enzymes are used in many areas for their beneficial effects including the environment, agriculture, livestock, households and aquaculture.During the

production of eco-enzymes, catalase process generates ozone (O_3), which promotes the CO_2 reduction in the atmosphere and can trap heavy metals in the cloud clusters while reducing thus the effect of global warming. At the same time, nitrate (NO_3) and carbonate (CO_3) are formed to improve soil fertility and natural plants. Furthermore, they are used to purify the environment. Enzymes contained in the solution neutralize toxins and other pollutants from rivers, soils and atmospheres (Triassi, *et al.,* 2015).

Eco-enzymes are also used to disinfect water on farms, as food supplements to animals and to reduce odors from farms.International aqua feed (2012) reports that such enzymes have the ability to stabilize the soil organic matter and can be effectively used to ensure the quality of soil and farming conditions of aquatic species. The mixture that contains the variety of enzymes can be effective means for bioremediation in aquaculture.

Eco-enzymes have been used to accelerate the degradation of organic matter (feces,uneaten feed and dead algae), destroy the deposition of particles, reduce deposit accumulation and the solid contents, decompose plant debris, reduce anaerobic conditions, depths of the pond, promote the degradation of some complex nutrients and facilitate high nutrient digestibility(Madhumithah *et al.,* 2011).

The effectiveness of eco-enzyme is very important to manage waste in composting also minimize pest disturbance (Saravan *et al.,* 2013). According to research results, vegetable waste is very effective in the formation of volatile fatty acids (VFA) and nutrients such as nitrogen content that is useful for plants. The market waste management, especially vegetable and fruit bark waste used as eco garbage enzyme will be applied as an eco-friendly bio pesticide that will be used on hydroponic vegetable crop so as to realize healthy life style (Bo *et al.,* 2007).

Thus the aim of the present work is to explore the possibility of eco-enzyme to implement in all walks of life to achieve Go Green vision for waste disposal around the world and to provide a quality environment from pollution.

The main objectives of the study are stated below:

- To minimize the environmental pollution by the usage of organic wastes like fruits, vegetables and kitchen waste.
- To analyze the fermented eco enzyme in terms of physicochemical parameters.
- To investigate the performance of eco-enzyme in destroying harmful pathogens and also as a growth stimulator for both plants and animals.
- To test the larvicidal activity of the fermented eco-enzyme.
- To check the stain removing capacity of the eco enzyme on cotton fabrics.

2. REVIEW OF LITERATURE

The literature pertaining to the study entitled as "Potential implementations of eco enzyme" is reviewed under following headings:

2.1 SCENARIO OF LAND POLLUTION

2.2 PRODUCTION OF ECO-ENZYME FROM ORGANIC WASTES

2.3APPLICATIONS OF ECO-ENZYME

2.3.1 Antimicrobial agent

2.3.2 An effective larvicide

2.3.4 Detergent cleaner

2.1 SCENARIO OF LAND POLLUTION DUE TO ORGANIC WASTES

Environment pollution is a wide-reaching problem and it is likely to influence the health of human populations is great. This paper provides the insight view about the effects of environment pollution in the perspective of air pollution, water and land/soil waste pollution on human by diseases and problems, animals and trees/plants (Khan and Ghouri 2011).

A study was conducted to assess the seasonal variation in the quantity of generated municipal solid waste in Guwahati city. It revealed that food and vegetable waste is generated in highest quantity in Guwahati city than other components of solid wastes. Variation occurs in the type, nature and quantity of municipal solid wastes generated at different period of time in a year depending upon the seasonal and environmental impacts. Moreover, the amount of solid waste generation generally increases during festivals, fairs, social and family parties and other special occasions

The use and associated disposal of persistent organic pollutants (POPs) have been occurring for over 50 years. Concurrent with the phase-out of some of the most hazardous chemicals, the production of new POPs, such as brominated and fluorinated compounds has increased since the 1990s. These latter compounds are commonly used in a wide range of consumer goods, and as consumer products reach the end of their useful lives, ultimately enter waste

recycling and disposal systems, in particular at municipal landfills. Because of their very slow, or lack of degradability, POPs will persist in landfills for many decades and possibly centuries. Over these extended time periods engineered landfill systems and their liners are likely to degrade, thus posing a contemporary and future risk of releasing large contaminant loads to the environment (Ronald *et al.*, 2011)

Due to enhanced economic activities and rapid urbanization, waste generation has increased dramatically in the last few decades. Municipal solid waste management (MSWM) is a challenging problem for developing countries. India produces 42.0 million tons of municipal solid waste annually at present. Annual increase in overall quantity of solid waste is assessed at about 5% and nearly three-fourths of the waste is generated in urban areas. MSW amount is expected to increase significantly in the near future as the country strives to attain an industrialized nation status by the year 2020. Municipal Solid Waste (MSW) generation in Mumbai is highest being 5,355 (tpd) followed by Delhi and Kolkata being 4000 and 3692 (tpd) respectively. When solid waste is disposed off on land in open dumps or in improperly designed landfills (e.g., in low lying areas), it causes an adverse impact on the environment, such as ground water contamination, generation of inflammable gases, acidity to surrounding soil, release of greenhouse gases *etc*. (Ashfaq and Khatoon 1970).

Of the many sources of urban greenhouse gas (GHG) emissions, solid waste is the only one for which management decisions are undertaken primarily by municipal governments themselves and is hence often the largest component of cities' corporate inventories. It is essential that decision-makers select an appropriate quantification methodology and have an appreciation of methodological strengths and shortcomings. Mohareb, *et al.,* (2011) compared four different waste emissions quantification methods, including Intergovernmental Panel on Climate Change (IPCC) 1996 guidelines, IPCC 2006 guidelines, U.S. Environmental Protection Agency (EPA) Waste Reduction Model

(WARM), and the Federation of Canadian Municipalities-Partners for Climate Protection (FCM-PCP) quantification tool. Waste disposal data for the greater Toronto area (GTA) in 2005 are used for all methodologies; treatment options (including landfill, incineration, compost, and anaerobic digestion) are examined where available in methodologies. Landfill was shown to be the greatest source of GHG emissions, contributing more than three-quarters of total emissions associated with waste management. Results from the different landfill gas (LFG) quantification approaches ranged from an emissions source of 557 kt carbon dioxide equivalents (CO_2) (FCM-PCP) to a carbon sink of -53 kt CO_2 (EPA WARM).

Municipal solid waste is a significant contributor to greenhouse gas emissions through decomposition and life-cycle activities processes. The majority of these emissions are a result of landfilling, which remains the primary waste disposal strategy internationally. As a result, countries have been incorporating alternative forms of waste management strategies such as energy recovery from landfill gas capture, aerobic landfilling (aerox landfills), pre-composting of waste prior to landfilling, landfill capping and composting of the organic fraction of municipal solid waste. As the changing global climate has been one of the major environmental challenges facing the world today, there is an increasing need to understand the impact of waste management on greenhouse gas emissions. This review paper serves to provide an overview on the impact of landfilling (and its various alternatives) and composting on greenhouse gas emissions taking into account streamlined life cycle activities and the decomposition process. The review suggests greenhouse gas emissions from waste decomposition are considerably higher for landfills than composting. However, mixed results were found for greenhouse gas emissions for landfill and composting operational activities. Nonetheless, in general, net greenhouse gas emissions for landfills tend to be higher than that for composting facilities (Lou and Nair, 2009).

An analysis of the generation, composition and management of the urban solid waste in Mexico and its relation to greenhouse gas emissions is described; as well a case study in More- los, a state in the central region of the country. Data were collected from the scientific literature and existing data bases at state and national levels. In addition, the emissions of greenhouse gas were calculated for a period of 14 years, using the Intergovernmental Panel on Climate Change (IPCC) methodology. The municipal solid waste data collected from 1998 to 2012 reveal an increase in the amount of waste generated in Mexico and in Morelos (38% and 43%, respectively), which have been influenced by the urbanization process and the population increase. According to the official data, the composition of the urban solid waste in Mexico, is mostly organic matter (50%), represented by food and garden residues, as well as paper and cardboard (near to 14%). While in Morelos, the percentages of generation for these materials are 44% and 9%, respectively. The management of the urban waste mainly consists of house collection, principally in metropolitan zones and medium and small cities, representing 78.7% in Mexico and 89.2% in Morelos. The second way to eliminate the solid wastes is open burning (mostly in semi-urban and rural areas), representing 14.5% and 6.7% for Mexico and Morelos, respectively. During this period, the nationwide greenhouse gas emissions derived from solid waste management (SWM) increased by 180%, while in Morelos, an increase of 42.5% was calculated. Thus, the population increment and urbanization process were correlated with the rise in the amount of residues generated in Mexico and Morelos (Castrejon-Godinez *et al.,* 2015).

2.2 PRODUCTION OF ECO-ENZYME FROM ORGANIC WASTES

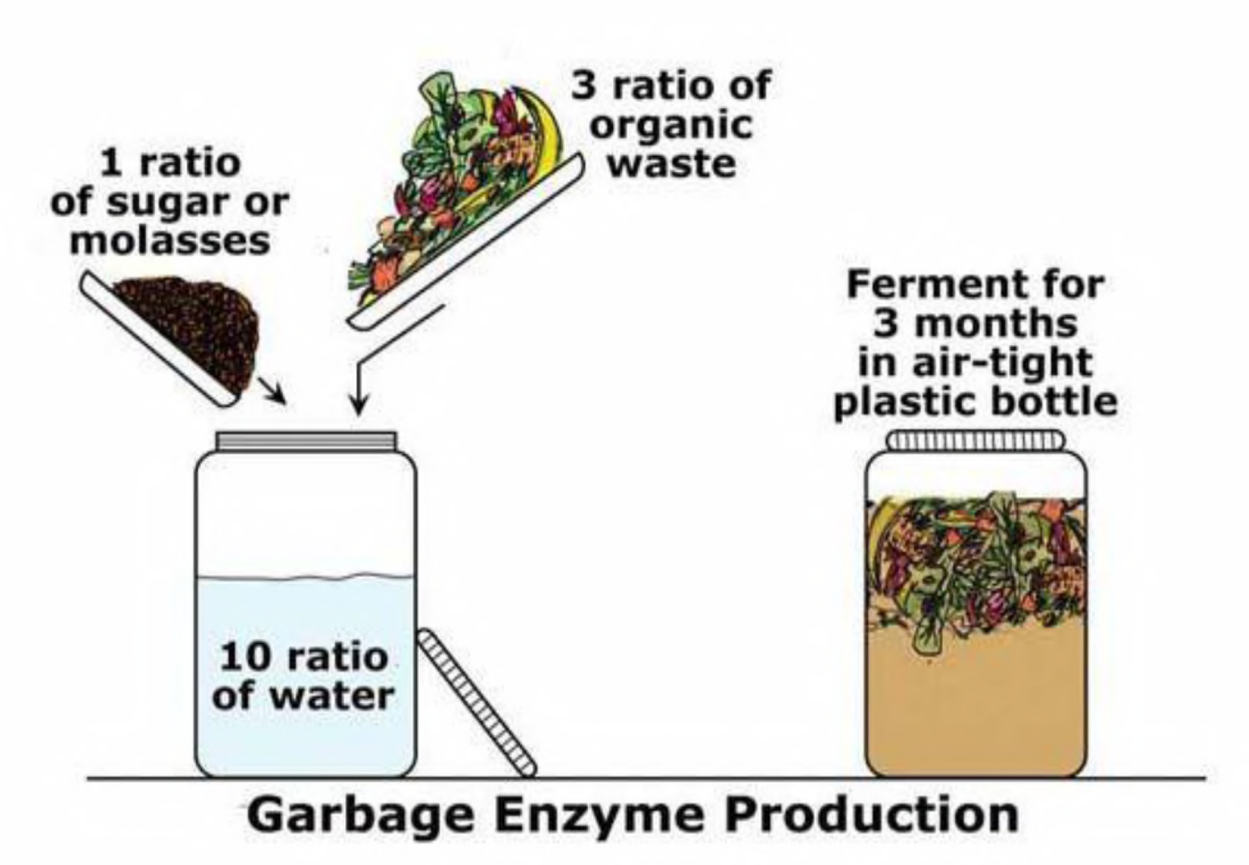
Eco enzyme is an organic solution produced by the simple fermentation of fresh vegetable waste, brown sugar and water, in much the same process that wine is made Fig.1 This fermentation creates natural chains of proteins, mineral salts and enzymes. This solution has the capacity to breakdown, change, create and catalyze functions that make it a wonderful cleaning aid. Garbage enzyme

solution was developed by Dr. Rosukon from Thailand. She has been actively involved in enzyme research for more than 30 years and encourages people to make garbage enzyme at home to ease global warming (Arun and Sivashanmugam, 2015).

Eco enzyme serves as a suitable alternate to the synthetic cleaning solution with regard to biodegradability, low toxicity, non-corrosiveness, environment –friendliness, enhanced cleaning properties as well as increased efficiency and stability in different environmental conditions are required to develop.

Fig. 1

Preparation of eco-enzyme



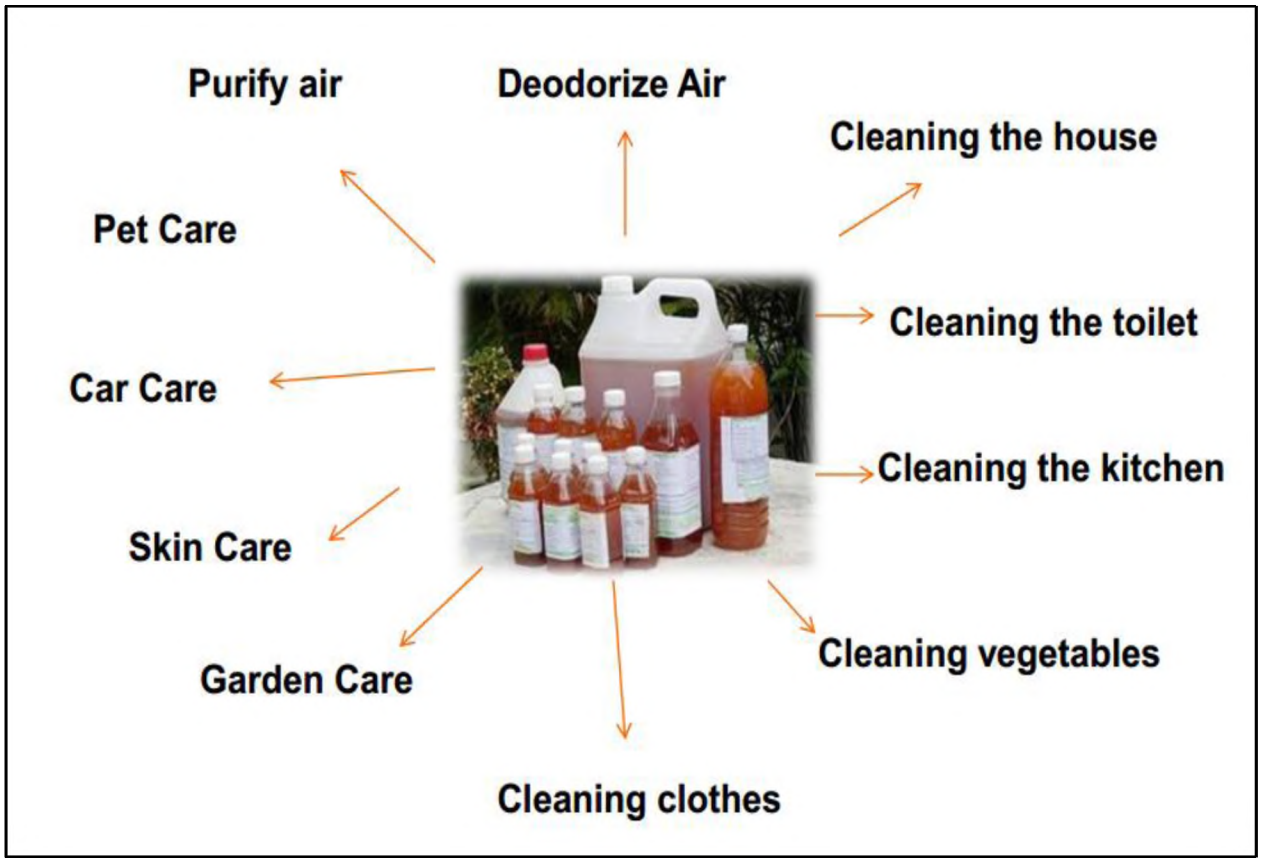
2.3 APPLICATIONS OF ECO-ENZYME

The advantages of using enzyme bio-cleaning solution are given below:

- ❖ It is safer for the environment and safer for human health than traditional chemical cleaners and odor control products.
- ❖ It is highly specialized enzyme producing microorganisms to clean and control odors by eliminating the soils rapidly.
- ❖ It is economically cheaper and the cost of production is less.
- ❖ These Bio-cleaning solutions provide residual cleaning for longer period and gives stable application.
- ❖ This Enzyme Bio-cleaning solution help to displace unknown, potentially pathogenic (disease causing) bacteria with known, healthy microorganisms and in this way contribute to better human health (Fig.2)

Fig. 2

Applications of eco-enzyme



2.4.1 Antimicrobial agent

The antimicrobial activity was done by agar well diffusion assay against five bacteria and three fungi. The citrus peel extracts showed highest zone of inhibition against pathogens, compared with the control Chloramphenicol and Griseofulvin used. Citrus peel extract showed good antimicrobial activity indicating its potency as a promising source of natural antimicrobials. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of the lemon peel (Arun and Sivashanmugam, 2015).

Fattouch *et al.,*(2007) evaluated the Quince (*Cydoniaoblonga* Miller) fruit aqueous acetone extracts. High-performance liquid chromatography−diode array detection and electrospray ionization−mass spectrometry were used for the identification and quantification of the phenolic compounds. The total phenolic content of the pulp and peel parts ranged from 37 to 47 and 105 to 157 mg/100 g of fresh weight, respectively. Chlorogenic acid (5-*O*-caffeoylquinic acid) was the most abundant phenolic compound in the pulp (37%), whereas rutin (quercetin 3-*O*-rutinoside) was the main one in the peel (36%). The radical scavenging potential of the extracts was determined and compared with that of synthetic antioxidants. The stronger properties corresponded to those obtained from peel material with a 70−80% inhibitory effect on DPPH radicals. The antimicrobial activity of the extracts against different microorganism strains was also investigated. Quince peel extract was the most active for inhibiting bacteria growth with minimum inhibitory and bactericide concentrations in the range of 10^2−5 × 10^3µg polyphenol /mL. It seems that chlorogenic acid acts in synergism with other components of the extracts to exhibit their total antimicrobial activities.

Pulp and peel aqueous acetone extracts obtained from Tunisian fruits at commercial maturity were comparatively evaluated for their phenolic profiles and antioxidant and antimicrobial potentials. The phenolic compounds present in the extracts were identified and quantified using RP-HPLC-DAD and ESI-MS techniques. Significant differences in the chromatographic profiles among these

fruits, as well as between pulp and peel extracts of each fruit, were observed. Quince, followed by 'Red Delicious', peel extracts showed the highest phenolic content (160.33 and 110.90 mg/100 g of fresh weight). The stronger inhibitory effect on DPPH radicals corresponded to those obtained from peel materials. A comparative analysis of the antimicrobial potential against a range of microorganism strains was also carried out. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus* were the most sensitive to the active extracts. Among the examined phenolic extracts, 'Red Delicious' and quince peels showed the highest effects for inhibiting bacteria growth. Minimum inhibitory and bactericide concentrations ranged from 10^2 to 10^4 µg of polyphenol/mL. Red skin apple and quince peels could be of great interest as important antioxidant and antimicrobial polyphenol sources (Cabboni *et al.,* 2008).

2.4.2 Feed for fishes

Aquaculture has developed through improved farming techniques, food quality and availability at lower cost. Through thedevelopment of new approaches to improving food, eco-enzymes have been incorporated into the Nile Tilapia feed (Oreochromis niloticus) to evaluate their effects. The objectives are the valuation of plant residues, the production of good quality feed and lowcost, and the analysis of the effects of eco-enzymes on Nile tilapia growth and survival, and improved digestibility. Thecompletion of this study was to produce eco-enzymes; then to make five (5) diets A, B, C, D, and E iso-protein (30%) containing respectively 0, 2.5, 5, 7.5 and 10% eco-enzymes. The experiment lasted two (2) months and was conducted in an isolated systemconsisting of5 treatments with 2 repetitions on tilapia fingerlings of 4.54 ± 0.3 g fed twice daily. Every two weeks fish wasweighed to monitor trends. Samplesof fish dorsal muscle before and after experiment were made for carcass compositionanalysis. The results showed that the diet C exhibited better weight gain 151.23% compared to the control127.75%; better TCS1.67% / d compared to control

1.50% / d and increased TCA is 3.68 against 4.12 in control. Moreover, the results showed thatthediet D had better survival90% against 45% in control. In short, eco-enzymes have played an important role in improving diet,tilapia growth and survival. Increasing the amino acid profile can do improving the nutritional quality of the food perspective.Also, eco-enzymes could beused to strengthen the immune system of Nile tilapia (Tokpohozin *et al*., 2015).

2.4.3 An effective larvicide for mosquito larvae

Management of mosquito vectors by current classes of mosquitocides is relatively ineffective and necessitates prospecting for novel insecticides with different modes of action. Larvicidal activities of 15 crude extracts from three geographically isolated *Aloe ngongensis* (Christian), *Aloe turkanensis* (Christian), and *Aloe fibrosa* (Lavranos&L.E.Newton) (Xanthorrhoeaceae) species were evaluated against *Aedesaegypti* (Linnaeus *in*Hasselquist) (Diptera: Culiciade L.) yellow fever mosquito. Freshly collected leaves were separately shade-dried to constant weight at room temperature ($25 \pm 2°C$) and powdered. Each powder was macerated in solvents of increasing polarity (hexane, chloroform, ethyl acetate, acetone, and methanol) for 72 h and subsequently filtered. Third-instar larvae ($n = 25$) of the mosquito were exposed to the extracts at different concentrations for 24 h to establish dose response relationships. All the fractions of *A. ngongensis* were active below 1 mg/ml except *A. fibrosa* and *A. turkanensis.* The highest activity (LC_{50}) mg/ml was obtained with extracts of *A. fibrosa* hexane (0.05 [0.04–0.06]), followed by *A. ngongensis* hexane (0.11 [0.08–0.15]) and *A. turkanensis* ethyl acetate (0.11 [0.09–0.12]). The activities are apparently *Aloe* species specific and extraction solvent dependent. (Chore *et al., 2014)*.

Mosquitoes are the carriers of severe and well-known illnesses such as malaria, arboviral encephalitis, dengue fever, chikunguniya fever, West Nile virus and yellow fever. These diseases produce significant morbidity and mortality in humans and livestock around the world. Murugan *et.al.,* in 2012 studied the

effects of orange peel ethanol extract of *Citrus sinensis* on larvicidal, pupicidal, repellent and adulticidal activity against *Anopheles stephensi*, *Aedesaegypti* and *Culex quinquefasciatus*.

Govindarajan and Sivakumar 2012 have been made an attempt to evaluate the combined effect of *Clerodendroninerme* and *Acanthus ilicifolius*on three species of mosquito vectors, Anopheles stephensi, *Aedes aegypti* and *Culex quinquefasciatus*. Different concentrations of *Clerodendroninerme* and *Acanthus ilicifolius* have been tested on the various stages ofspecies of mosquito vectors. Lethal concentrations (LC50 and LC90) were also worked for the different larvalstages of mosquitoes. Significant increased mortality was evident after the plant extracts. The lethal effect onmosquito larvae may be due to the active plant compounds on the gut lining of the mosquito larvae. Thelarval density was decreased after the treatment of plant extracts at the breeding sites (drinking water and ditches water).

2.4.4 Enzymes as a cleaning agent.

Parkar *et al*. (2004) stated that the cleaning strategies tested were based on biofilm biochemistry and physiology, and focused on the chemistry of the cleaners, the duration and temperature of the cleaning process and a combination of various cleaners. The success of the cleaning regimes was determined based on the removal of cells and organic debris and the elimination of viable cells. The results confirmed that a caustic and acid (75_C for30 min) wash, relied upon heavily in most food processing industries for cleaning-in-place systems and were successful in removing these biofilms.

Jegannathan *et al*., (2013) stated that the enzymatic processes have been implemented in a broad range of industries in recent decades because they are specific, fast in action and often several raw materials of energy ,chemical lsandor water compared to conventional processes. A number of comparative environmental assessment studies have been conducted in the past 15 years to investigate whether these properties of enzymatic processes lead to

environmental improvements and assess whether they could play a role in moving toward cleaner industrial production.

Enzymes have effectively assisted the development and improvement of modern household and industrial detergents. The major classes of detergent enzymes—proteases, lipases, amylases, and cellulases—each provide specific benefits for application in laundry and automatic dishwashing. Historically, proteases were first to be used extensively in laundry detergents. In addition to raising the level of cleaning, they have also provided environmental benefits by reducing energy consumption through shorter washing times, lower washing temperatures, and reduced water consumption. Today proteases are joined by lipases and amylases in improving detergent efficacy especially for household laundering at lower temperatures and, in industrial cleaning operations, at lower pH levels. Cellulases contribute to overall fabric care by rejuvenating or maintaining the new appearance of washed garments. Enzymes are produced by fermentation technologies that utilize renewable resources.

Hasan *et al.*, (2010) explained that the microbial lipases are an important group of biotechnologically valuable enzymes,because of the versatility of their applied properties and ease of mass production. Lipases of microbial origin are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications.Enzymes can reduce the environmental load of detergent products as the chemicals used in conventional detergents are reduced; they are biodegradable, non-toxic and leave no harmful residues. Besides lipases, other enzymes are widely used in household cleaning products, in laundering, medical, agriculture, etc.

Silva *et al.*, (2011) explained an enzymatic treatment is proposed as a preparative, cleaning protocol to remove cellulose films from resonators and sensors. Quartz crystal and surface Plasmon gold sensors, coated with ultrathin films of cellulose are used in studies of molecular adsorption. The sensors are usually recycled after removal of the film, with limited success, after one of two

treatments, either hot acid or ammoniac solutions. In the proposed, improved protocol a mixture of cellulases from *Aspergillus* species, are used as a pre-treatment to facilitate the release of the cellulose film from the surfaces of the sensors. It is concluded that the use of the recycled ammoniac cleaning solution after the enzymatic treatment is a very convenient, safe and less time consuming way to remove the cellulose films from the sensors to be recycled.

Prasad rao *et al*., (2014) explained that pectinase enzyme was produced from *Aspergillus niger* NCIM 548 under solid state fermentation (SSF) using agriculture residue and horticulture wastes. Sixteen substrates were screened for pectinase production of which jack fruit waste was found to be the best substrate. The maximum yield of pectinase 39.836U/gds was obtained with jack fruit waste 10g, particle size 152-354 µm, moisture content 70%v/w, pH 5.0, temperature 30oC, glucose 3.5%w/w, (NH)4SO4 1.0% w/w and fermentation time72 h.

Howard *et al*., (2003) stated the related areas of lignocellulose research of the enormous economic potential of the bioprocessing of residual plant materials generally regarded as “waste”, and secondly to highlight some of the modern approaches which potentially could be used to tackle one of the major impediments, namely high enzyme cost, to speed-up the extensive commercialisation of the lignocellulose bioprocessing.

Samanta *et al*., (2012) stated that bio surfactants are surface-active substances synthesized by microorganisms having the properties of reducing surface tension, stabilizing emulsions, promoting foaming and are generally non-toxic and biodegradable. Here an effort was made to screen biosurfactant activity of a protease producing bacteria isolated from municipal solid waste. Strain was identified as *Pseudomonas aeruginosa* by 16S rDNA based molecular technique. Biosurfactant, obtained from isolated organism was screened by hemolytic assay,drop collapsing method, oil spread method, blue agar plate method and oil spreading technique. Besides biosurfactant activity the strain also produces protease enzyme. The strain has shown maximum protease activity at pH 9.5,

temperature 37°C and 48 hrs. of incubation time. So, this strain can be used in textile, leather, detergent, pharmaceutical and dairy industries for its dual ability of producing protease enzyme and biosurfactant activity.

Renge *et al.*, (2012) stated that the enzymes are proteins, which act as catalysts. Enzymes lower the energy required for a reaction to occur, without being used up in the reaction. Many types of industries, to aid in the generation of their products, utilize enzymes. Examples of these products are; cheese, alcohol and bread. Fermentation is a method of generating enzymes for industrial purposes. Fermentation involves the use of microorganisms like bacteria and yeast to produce the enzymes. There are two methods of fermentation used to produce enzymes. These are submerged fermentation and solid-state fermentation. Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Solid-state fermentation is the cultivation of microorganisms, and hence enzymes on a solid substrate. Carbon containing compounds in or on the substrate are broken down by the microorganisms, which produce the enzymes either intracellular or extracellular.

The enzymes are recovered by methods such as centrifugation, for extracellularly produced enzymes and lysing of cells for intracellular enzymes. Many industries are dependent on enzymes for the production of their goods. Industries that use enzymes generated by fermentation are the brewing, wine making, baking and cheese making.

Chancharoonponga *et al.* (2012) stated that soybean koji is an important ingredient for traditional fermented food in South-East Asia and East Asia. Koji containing 60% soybean was used as substrate to investigate the enzyme production by *A. oryzae*. During koji fermentation, pH increase of soybean koji was caused by enzymes production. The highest protease and amylase activities were 84.38 and 200 unit/g of dry weight, respectively. Moreover, growing of enzyme activities on soybean koji correlated with the growth of this mold.

Electron micrograph showed that spores of A.oryzae S. were formed after 48 h of cultivation period.

Carlos Regalado *et al.*, (2010) described that Hemicellulosic agricultural byproducts such as corn stover (CS) are highly available materials which represent an opportunity to develop value added products. Native *Aspergillusniger*GS1 was used for solid-state fermentation (SSF) on alkali pre-treated CS (ACS) aimed to optimize xylanolytic enzymes production, and their effect on *in vitro* ruminal and true digestibility of ACS. CS is a readily available by-product in different regions which after alkaline treatment and partial hydrolysis with the EE, may be advantageously used as supplement for ruminant feed.

Toca-Herrera *et al.*, (2007) explained that solid-state fermentation (SSF) processes involve the growth of microorganisms (typically fungi) on a solid material in the absence or near absence of free-flowing water. Utilization of agro-industrial residues as support-substrates in SSF processes provides an alternative avenue and value-addition to these otherwise under- or non-utilized residues. SSF processes have shown to be particularly suitable for the production of enzymes by filamentous fungi, since they reproduce the natural living conditions of such fungi.

Unakal *et al.*, (2012) explained Banana waste can be used as a substrate for the production of amylase by *Bacillus subtilis* using solid state fermentation with various process parameters like, the incubation period, substrate concentration, pH and incubation temperature showed 24hrs, 50g, 7 pH and 35°C respectively. Peptone (0.2%) as a nitrogen sources showed maximum yield and the maximum enzyme activity showed in presence of in organic nutrients magnesium sulphate ($MgSO_4.7H_2O$), calcium chloride ($CaCl_2.2H_2O$) and di-hydrogen potassium phosphate (KH_2PO_4) were 0.02%, 0.04% and 0.4% respectively.

Hostinova *et al.*, (2002) studied two strains of the food-borne amylolytic yeast *Saccharomycopsis buligera* with respect to production and characterisation of their amylolytic enzymes. *S.buligera* K_Z represents a strain synthesizing an amylolytic complex composed of amylase, glucoamylase and glucosidase. *S. buligera* IFO 0111 represents a strain producing only oneamylolytic enzyme glucoamylase, with a property unique among yeast amylases, namely theability to degrade raw starch.

Dezsi *et al.*, (2011) explained the influence of vitamins and zinc acetate on the synthesis of the enzyme invertase by nine yeast strains belonging to the genus *Saccharomyces*, namely species *S. carlsbergensis (*beer yeast), *S. cerevisiae*(bread yeast) and *Sacch. Ellipsoideus* (wine yeast) investigated by CsillaKatalindezsi. As invertase producer, the yeast SCHCCBM 307 (from the Biotechnology andMicrobiology Research Center at Lucian Blaga University in Sibiu) was the best on the control substrate (malt wort) and on the substrate enriched with both vitamins acetate and the yeast SEJ 103 (from the Jidvei Center) was the best on media enriched only withvitamins.

Loc *et al.*, (2011) explained the production of neutral protease (NPRC10) by recombinant E. coli BL21 (DE3) through submerged culture in 40-L fermenter with working volume of 20 L. The parameters such as cell density, pH, inoculum size, and agitation speed were investigated for the production of enzyme. The results shown that the maximum production of NPRC10 was obtained after 34 h of batch fermentation at OD600 (cell density) of 2, inoculum size of 2% and agitation speed of 500 rpm with medium pH maintained at 7. The highest total activity of NPRC10 during the course of fermentation was approximately 76 unit/ml.

Nayak *et al.*, (2011) explained the thermostable properties of *Taq*DNA polymerase from *Thermusaquaticus* have contributed greatly to the yield, specificity, automation, and utility of the polymerase chain reaction method for amplifying DNA investigated by Nayak. *Taq*polymerase is widely used enzyme

for DNA amplification in PCR techniques and highly applicable in molecular biology and biotechnology. More than 50 DNA polymerase genes have been cloned and sequenced from various organisms including thermophiles by PCR cloning technique, whereby the gene encoding this enzyme was cloned into the expression vectors that produce recombinant *Taq*polymerase gene has facilitated for this enzyme production.

Javadpour *et al*., (2002) described that Penicillin amylase (EC 3.5.1.11) has been a target of study for a long time because of its pivotal role in the deacylation of the penicillin into the 6- aminopenicillanic acid (6-APA) and the side-chain organic acids. In this study, Sixty-five strains of *E. coli* were investigated for penicillin acylase activity using fluorescamine method.

Javadpour *et al*., (2002) stated that cellulase is a group of enzymes (endoglucanase, exoglucanase and betaglucosidase) required for cellulosic feedstock hydrolysis during bioethanol production by Andre L. Rodrigues. The use of recombinant cellulase is a strategy to reduce the enzyme cost. In this context, the present work describes the construction of a cellulase expression vector (pEglABglA), which allowed constitutive co-expression of endoglucanase A (EglA) from an endophytic *Bacillus pumilus* and the hyperthermophilic β-glucosidase A (BglA) from *Fervidobacterium* sp. in *Escherichia coli.*

Janarthanan *et al*., (2014) described that the Urbanization and industrializationaccompanied by population flare-up has formed a serious problem of waste generation and its disposal, treatment and management. The solid wastes are generated more in some parts Salem city, Tamil Nadu. In the present study, the vegetable wastes were collected from various sources like vegetable market, reception halls, hospitals, schools and market areas which were mainly from Ammapet, Hasthampatti, Suramangalam and Kondalampatti regionat Salem district. In this study, there are 40 different bacterial strains were isolated and identified. Among the strains, *Bacillus* sp. (B17), *Micrococcus* sp. (C3), and *Bacillus* sp. (P1) were identified as efficient starch hydrolyser and

those were completely composted the market waste in very short duration when compared to the normal soil micro flora. The amylase enzyme assay also checked by Dinitrosalicylic Acid (DNS) method. In compost the NPK level was increased significantly and it could be helpful for the plant growth. In pot culture study, very lesser application of compost (2:1 - soil: compost) showed best results.

Madhumithah *et al.*, (2011) study was taken up to utilize different vegetable wastes as input for protease production using *Aspergillus niger.* Wastes like potato, pumpkin, cauliflower, cabbage and brinjal procured from local market served as substrates for the solid state fermentation. It is a novel, economical approach for the bioconversion of vegetable wastes for the production of protease that is industrially significant.

Saravanan *et al.*, (2012) described the optimization of the media components for cellulase production using *Trichoderma reesei*. The optimization of cellulose production usingpineapple waste as substrate was performed with statistical methodology based on experimental designs. The screening of nutrients and their influence on the cellulose production was studied using a Plackett-Burman design. Avicel, soybean cake flour, KH PO, and yeast extract were found to have the positive influence for the production of cellulase.

Saheed *et al.*, (2013) explained the white rot fungus arevaluable class of filamentous and spore forming strains capable of use as animal feed supplements when cultivated under submerged state bioconversion. Selected bacidiomycetes; *Phanerochaete chrysosporium, Panus tigrinus* M609RQY (M6) and RO2 were grown solely on liquid and solid substrates of banana peel, pineapple peel and papaya peel. While *P.chrysosporium* synthesized 8.16 and 10.21 mg g1. *P. chrysosporium*, M6 and RO2 produced good αamylase and cellulase enzyme activities that assisted in substrate degradation for protein synthesis.

Rahna *et al.*, (2011) explained reduced production cost of cellulase by using alternative carbon source such as lignocellulosic waste and optimized fermentation parameters for high yielding. In the present investigation, isolated the novel cellulase producing actinomycetes, *Streptomyces sp* from decayed fruit waste and optimized the physicochemical parameters for cellulose production. It could be concluded that *Streptomyces sp S7* is a powerful cellulase producer strain under our tested experimental conditions using fruit waste as carbon source.

3. MATERIALS AND METHODS

The methodology pertainingfor the present study entitled "*Potential Implementations of Eco-enzyme"* was described under the following headings:

3.1 Collection of fruit wastes

3.2 Fermentation of fruit wastes

3.3 Characterization of eco-enzyme

3.4 Enzymatic analysis

- 3.4.1 Test for Amylase
- 3.4.2 Test for Maltase
- 3.4.3 Test for Invertase
- 3.4.4 Test for Protease

3.5 Antimicrobial activity of eco-enzyme

3.6 Impact of eco-enzyme (raw and diluted) on the biometric parameters of green gram

- 3.6.1 Collection of the soil sample
- 3.6.2 Selection of the experimental plant
- 3.6.3 Pot culture experiments
- 3.6.4 Biometric measurements

3.7 Impact of eco-enzyme on the growth of the fish species *Catla catla*

- 3.7.1 Experimental fish
- 3.7.2 Growth parameters

3.8 Larvicidal activity of eco-enzyme

3.9 Impact of eco-enzyme in removing methyl red stain on fabric

3.1COLLECTION OF FRUIT WASTE

Fruit wastes such as watermelon, mosambi, pineapple, and pomegranate were collected from Saradalaya fresh juice stall located in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu. The collected wastes were brought to the laboratory using sterile plastic bags for further process. Those wastes were then cut into small pieces with the help of knife for fermentation (Plate 1).

3.2 FERMENTATION OF FRUIT WASTES

The fruit waste was weighed using electronic weighing balance. After weighing, Brown sugar,fruit waste and water were added in an air tight plastic container in the 1:3:10and mixedthoroughly.The mixture was allowed to fermentate for a period of three months (Plate 2). After fermentation the solution was filtered to separate it from solid residues and used as eco enzyme source.

PLATE 1

Fruit wastes selected for the study

PLATE 2

Fermentation of fruit wastes

3.3 CHARACTERISATION OF ECO-ENZYME

3.3.1 Analysis of physical parameters

The analysis was performed by following the standard method of APHA, (1992).

Colour

The colour of the sample was visually observed.

Odour

The odour of the sample was noted by directly smelling the sample.

Temperature

Temperature was measured after the fermentation period , using mercury filled centigrade thermometer (0º C to 50º C). The readings were made by dipping the thermometer in water for 2 minutes before constant readings were obtained.

Electrical conductivity

The electrical conductivity was estimated using conductivity bridge and expressed in µs.

Total Dissolved Solids

50 ml of the sample was taken in a pre-weighed silica crucible and the sample was evaporated to dryness using a water bath. After complete evaporation the final weight of the crucible was taken. The total dissolved solids present in the sample was calculated by using the following formula

$$\text{TDS(mg/l)} = \frac{\text{Final wt.} - \text{Initial wt. of the crucible}}{\text{Volume of the sample}}$$

3.3.2Analysis of chemical parameters

pH

A direct reading pH meter was used. The pH meter was first standardized using buffer solutions of pH 7.0 and pH 9.2. The electrodes were rinsed in distilled water and immersed in the water samples and readings were noted in the digital display.

Dissolved oxygen

Dissolved oxygen of the water sample was estimated by Winkler's method.

Estimation of Biochemical Oxygen Demand (BOD)

Reagents

Phosphate buffer solution

33.4g of disodium hydrogen phosphate, 8.5g of potassium dihydrogen phosphate, 21.75 g of dipotassium hydrogen phosphate, 1.7g of ammonium chloride was dissolved in 1000 ml of distilled water in a volumetric flask and the pH was adjusted to 7.2.

Dilution Water

Double distilled water taken in a glass container was aerated for half an hour using an aerator. 1 ml of phosphate buffer, 1 ml of M_9SO_4 (22.5 g/l), 1 ml of $CaCl_2$ (27.5 g/l) and 1 ml of $FeCl_3$ (0.25 g/l) were added.

Chemicals needed for DO estimation (correction)

Procedure

1. The sample was diluted (measured dilution) with dilution water.

2. The sample was taken in two BOD bottles. D.O content (D1) of one bottle was analyzed and the other was incubated in BOD incubator at 20º C for 5 days.
3. Two other bottles were filled with dilution water D.O content was analyzed immediately in one bottle and the other was incubated.
4. D.O was analyzed in the incubated water sample (D2) and dilution water after 5 days of incubation.

Calculation

$$\text{BOD (mg/l)} = \frac{(D1 - D2 - BC) \times 100}{\text{Percentage dilution of sample}}$$

BC – Blank correction

Estimation of Chemical Oxygen Demand (COD)

Reagents

0.25 N Potassium dichromate

12.259 g of potassium dichromate in 1000 ml of distilled water.

0.1 N Ferrous ammonium sulphate (FAS)

39.2 g of ferrous ammonium sulphate and 20 ml of conc. H_2SO_4 was dissolved in 1000 ml of distilled water. The solution was standardized with 0.25 N potassium dichromate solutions.

Ferroin indicator

1.485 g of phenanthroline and 0.695 g of ferrous sulphate dissolved in 100 ml distilled water.

Procedure

1. 10 ml of sample was taken in a COD flask and 30 ml of conc. H_2SO_4 and 10 ml of 0.25 N potassium dichromate were added.
2. The content was refluxed for two hours in a hot plate at 60º C, then cooled, diluted with distilled water and made up to 140 ml.
3. Two to three drops of ferroin indicator was added and titrated against 0.1 N Ferrous ammonium sulphate.
4. The colour change from blue green to reddish brown was the end point. The entire procedure was repeated for blank.

   COD of the sample was calculated using the formula.

$$\text{COD (mg/l)} = \frac{\text{V X Normality of FAS X 8 X 1000}}{\text{Volume of the sample}}$$

3.4 Enzyme Analysis

3.4.1 Test for Amylase

To 2ml of the eco-enzyme, 2ml of 1% starch was added and the kept in boiling water bath for 30minutes.A drop of iodine was added to the test tube. Formation of dark blue colour solution indicates the presence of amylase.

3.4.2 Test for Maltase

To 2ml of the eco-enzyme, 2ml of 1% starch followed by 2ml of Benedicts reagentwas added and heated gently.The test tubes were placed in water bath for about an hour. Formation of reddish brown precipitate indicates the presence of maltase.

3.4.3 Test for Invertase

To 1ml of 5% sucrose, 3drops of eco-enzyme was added and kept in water bath for an hour.Then a drop of Fehling solution A and B were added and again kept in water bath for an hour.Formation of greenish blue colour indicates the presence of invertase.

3.4.4 Test for Protease

The eco-enzyme was treated with equal volume of 40% sodium hydroxide and two drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of protease.

3.5 ANTIMICROBIAL ACTIVITY OF ECO-ENZYME

The antibacterial and antifungal activity of the fermented eco-enzyme was assessed by standard agar well diffusion method (Bauer *et al*., 1996).The bacterial (*Pseudomonas aeurginosa, Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes)* and fungal cultures (*Aspergillusniger, Aspergillusflavus, Rhizopussp,Alterneriasp,Trichodermaviridae*) were used in the present study. SterileMuller Hinton Agar and Rose Bengal Chloramphenicol Agar mediawas poured on to the plates and allowed to solidify.A single well was bored on the agar plates using cork (0.6cm diameter). The culture of each isolate was swabbed uniformly onto the individual plates. 20µl of eco-enzyme was added into the well. Then the plates were incubated for 24 h at 37°C (Bacteria) and at room temperature for 5 days (Fungi). After incubation, different zone of inhibition in mm formed around the well were measured and expressed in millimeter.

3.6 IMPACT OF ECO-ENZYME ON THE BIOMETRIC PARAMETERS OFGREEN GRAM

A pilot study was carried out using different concentrations (0.1% - 1%) of eco-enzyme. It was found that 0.5% found to exhibit good activity and hence further study was carried out in this dilution.

3.6.1 Collections of soil sample

Red soil and sand for the pot culture experiment were collected from Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The soil sample was sieved and mixed with sand in equal proportion

3.6.2 Selection of the experimental plant

Green gram was selected for the present study. The plant is well suited to cool and temperate growing regions with low to moderate rainfall.

3.6.3 Pot culture experiments

Seeds were collected from local markets of Coimbatore. Six pots were set for the present investigation. Seven healthy seeds were sown in each pot and were kept under laboratory conditions. The plants were grown using fermented eco-enzyme at 0.5% dilution (T_1) and tap water (T_2) which served as control. Each treatment was replicated thrice. They were watered twice a day. The plants were uprooted on 7^{th} day and the biometric parameters such as germination percentage, vigour index, shoot length and root length were analyzed.

3.6.4 Biometric measurements

Germination percentage

After 7 days of sowing, germination percentage of the seedlings were calculated using the formula

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

The protrusion of radical through seed coat was taken as the criterion of germination.

Shoot length

The maximum length of each shoot was recorded in cm from the ground level to the tip. The plants were uprooted during the 7thday after sowing and washed in running water to remove soil particles and pressed between filter paper folds to remove water droplets before the shoot length was measured.

Root length

The plants were uprooted on 7th day taking utmost care not to damage the roots as far as possible. The maximum length of root was recorded in cm.

Vigour index

Vigour index was calculated as the product of germination percentage and plant height. The vigour index of each seedling was calculated using the formula

Vigour index= germination percentage X (root length + shoot length)

(Abdul Baki and Anderson, 1973)

3.7 IMPACT OF ECO-ENZYME ON THE GROWTH OF *Catla catla*

A pilot study was carried out using different concentrations (0.1%-1%) of eco enzyme. It wasfound that 0.5% found to exhibit good activity and hence further study was carried out in this dilution.

3.7.1 Experimental Fish

Fingerlings of *Catla catla*, fresh water edible fish were collected from fish farm in Aliyar dam. Fingerlings were transported in air tight bag and brought to the laboratory. Those fingerlings were maintained in large aquarium tank for a

week with required aeration and were acclimatized to the laboratory conditions. During the period of acclimatization fish were fed with rice bran and oil cake (Fish food) and water was changed at regular intervals to ensure sufficient oxygen supply to fish

3.7.2 Growth parameters

The experiment was carried out for 60 days with two treatments.Each trough contained eight fishes. The treatments were carried out in triplicates.

The weight and length of the control and experimental fishes were taken just before starting the experiment, followed by 30th day and 60th day. The growth parameters in terms of weight gain and length gain were evaluated as follows:

$$\text{Weight gain \%} = \frac{\text{Final weight (gm.) - Initial weight (gm.)}}{\text{Initial weight(gm.)}} \text{ X } 100$$

$$\text{Length gain \%} = \frac{\text{Final length (cm) - Initial length (cm)}}{\text{Initial length (cm)}} \text{ X } 100$$

3.8 ANIT-LARVICIDAL ACTIVITY OF ECO-ENZYME

3.8.1 Collection of Mosquito larvae

The *Culex quinquefciatus* Mosquito larvae was collected from stagnant waters along the roadsides of TVS, Coimbatore,Tamilnadu. The collected larvae were brought to the laboratory using sterile plastic bags for further process.

3.8.2 Experimental Set up

In the experiment, the larvae of *Culex quinquefciatus* was treated with different concentrations (5%,10%,15%,20%) concentrations of eco-enzyme and a control was maintained. Each treatment contained 20 larvae and the set upwas

kept undisturbed for 12hrs.After 12hrs, their mortality percentage was calculated using the formula given below:

$$\text{Mortality percentage} = \frac{\text{Number of larvae dead}}{\text{Total number of larvae}} \times 100$$

3.9 Impact of eco-enzyme in removing methyl red stain on fabric

Two pieces of 5cmX5cm cotton fabric was taken for the experiment. Both the pieces were dipped in methyl red stain for 5mins. After 5mins, one fabric was taken and immersed in water for 15mins which served as control, whereas the another fabric was taken and immersed in raw eco-enzyme for a period of 15mins. Then difference in colour was compared with the control and eco-enzyme treated fabric.

4. RESULTS AND DISCUSSIONS

Fruits and vegetable wastes are produced in large quantities in markets and constitute a source of municipal waste because of their high degradability. Instead of taking serious measures to decompose these type of wastes in soil, filling and dumping in the environment, the way of recycling the biological waste will give energy as well as prevent the environmental pollution. There are many methods of recycling agricultural wastes which are available to reuse the waste resources. One such method is making use of fruit and vegetable wastes to produce eco-enzymes and organic acids by simple fermentation process. The scientific way of doing the above eco-enzyme from microbial organisms in the fruit and vegetable wastes to make pollution free environment is the key perception of the project.

The results of the present study entitled "Potential implementations of eco-enzyme" are discussed under the following headings:

4.1 Fermentation of fruit wastes

4.2 Characterization of naturally synthesized eco-enzyme

4.3 Enzymatic analysis of the fermented eco-enzyme

4.4 Antimicrobial activity of the eco-enzyme

4.5 Impact of eco-enzyme (raw and diluted) on the biometric parameters of green gram

4.6 Impact of eco-enzyme on the growth the fish species *Catla catla*

4.7 Larvicidal activity of the eco enzyme

4.8 Impact of eco-enzyme in removing methyl red stain on fabric

4.1 FERMENTATION OF FRUIT WASTES

Eco-enzyme was prepared using 3 parts of waste mixture, 1 part of brown sugar and 10 parts of water in an air tight container. After three months, the fermented solution was filtered from solid residues and the brown coloured liquid was used as eco-enzyme(Plate 3). As the unwanted or the disposed wastes are reused the waste can be reduced significantly by preparing eco-enzyme. During the process of fermentation the ozone emitted can separate carbon-dioxide and other heavy metals from the air. As a result the heat trapped in the atmosphere will be reduced significantly.

PLATE 3

Production of eco-enzyme

4.2 CHARACTERIZATION OF NATURALLY SYNTHESIZED ECO-ENZYME

Physical parameters

The enzyme sample was pale yellow in colourand had a pungent vinegary odour. The temperature was recorded as 27.4ºC.Eco-enzyme can remain active even at a temperature above 100ºC when it is not exposed to air

The electrical conductivity of the enzyme was found to be 5.131(µs) and the total dissolved solids (TDS) was 4.696 ppm.

Chemical parameters

The pH value of the eco-enzyme was found to be 3.11. During fermentation, carbohydrates were converted into volatile acids and in addition, organic acids present in the waste material also leached out into the fermented solution. Hence the pH of the eco-enzyme was acidic in nature. The results obtained showed that the value of dissolved oxygen (DO) was 19.64 mg/l, the concentration of sodium chloride was 5.281 ppm, biological oxygen demand (BOD) was 81mg/l, and chemical oxygen demand(COD) was 153 mg/l respectively(Table 1).

TABLE 1

Physico-chemical parameters of the Eco-enzyme

S.No	Parameters	Values
1	Colour	Light to dark brown
2	Odour	Pungent vinegary smell
3	Electrical conductivity(µs)	34.81
4	Temperature(ºC)	27.4
6	Total dissolved solids(ppm)	4.696
7	pH	3.4
8	Dissolved oxygen(mg/l)	19.64
9	Biochemical oxygen demand(mg/l)	81.2
10	Chemical oxygen demand (mg/l)	153

4.3 ENZYMATIC SCREENING

The eco-enzyme was used to investigate the presence of various enzymes like amylase, maltase, invertase, and protease. The result of the enzyme analysis depicts the presence of amylase, maltase, invertase, and protease respectively.

4.4 ANTIMICROBIAL ACTIVITY

The antimicrobial potential of eco-enzyme against the selected bacterial (*Pseudomonas aeurginosa, Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes)* and fungal (*Aspergillusniger, Aspergillusflavus,Rhizopussp,Alterneriasp,Trichodermaviridae*) isolates were measured in terms of zone of inhibition. The results were depicted in table 2and plate 4 shows the Antibacterial and plate 5 antifungal activity of the eco-enzyme.

From the table 2 it was evident that the zone of inhibition against the raw eco-enzyme was recorded as 12mm,10mm, 11mm and 14mm for the *Pseudomonas aeurginosa, Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes.* Similarly for antifungal activity*Aspergillusniger, Aspergillusflavus,Rhizopussp,Alterneriasp andTrichodermaviridae* the zone of inhibition was observed as 7mm, 9mm, 10mm, 4mm and 5mm respectively.There was no zone of inhibition in 0.5% eco-enzyme which might be due to the diluted nature of the enzyme.

The results clearly revealed that the eco-enzyme has the highest power to inhibit the growth of bacteria and fungi due to its acidic nature which was generated during the fermentation of the organic waste materials (Prakash 2011). The extracellular enzymes which are produced during fermentation may be responsible for the lytic action and kills or inhibits the microbes. Also the

presence of acetic acid in the eco-enzyme serves to be a good antimicrobial agent. Eco-enzyme can also eliminate the harmful microbes and enhance cell regeneration.

TABLE 2

Antimicrobial activity of eco-enzyme

Microbial isolates	**Zone of inhibition (mm)**
Bacteria	
Pseudomonas aeurginosa	12
Staphylococcus aureus	10
Escherichia coli	11
Streptococcus pyogenes	14
Fungi	
Aspergillusniger	7
Aspergillusflavus	9
Rhizopussp	10
Alterneriasp	4
Trichodermaviridae	5

PLATE 4

Antibacterial activity of eco-enzyme

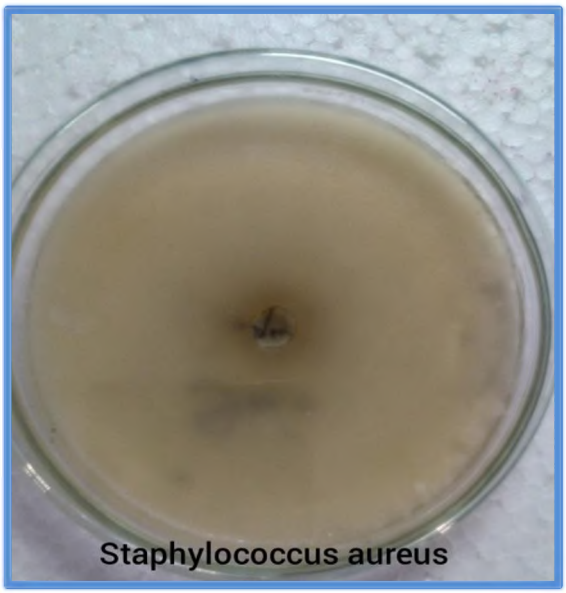
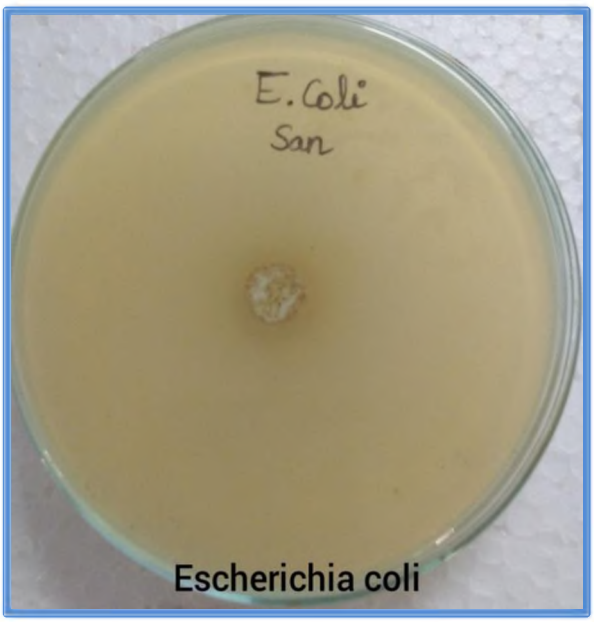
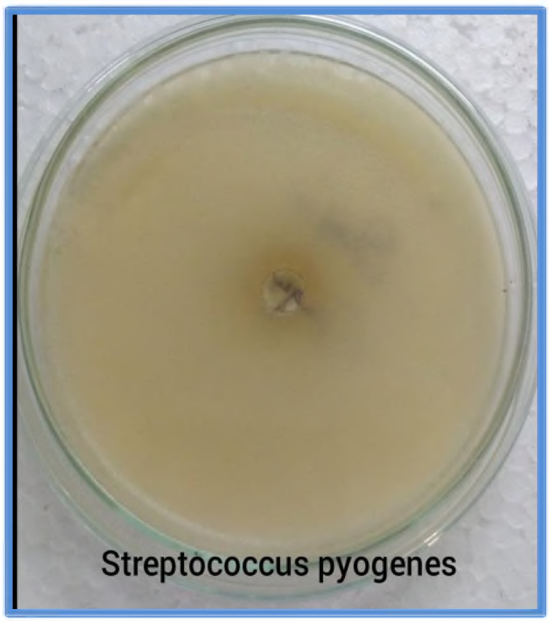
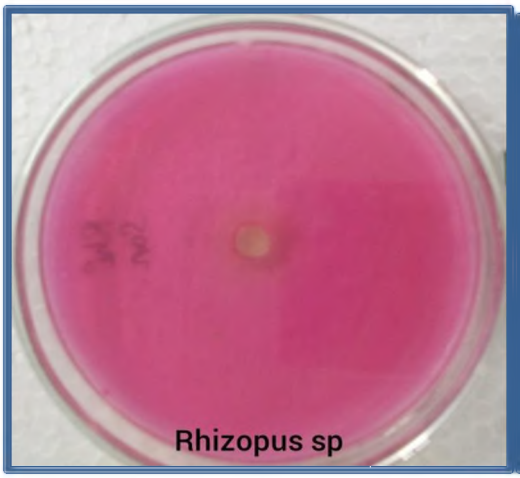


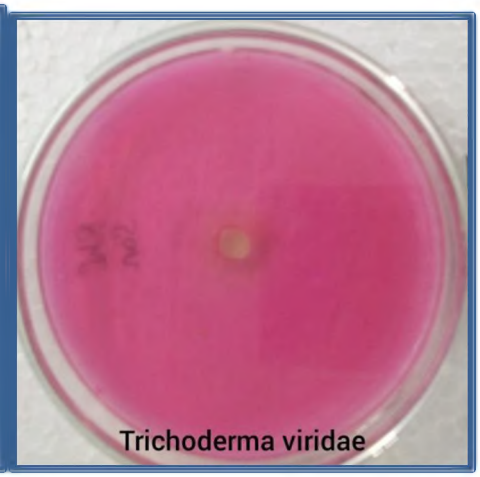
PLATE 5

Antifungal activity of eco-enzyme



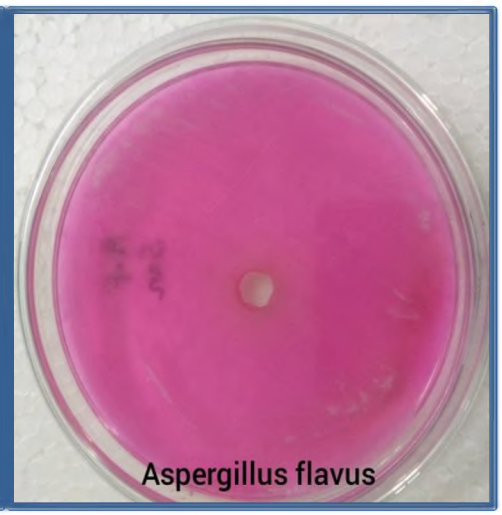



Rhizopus sp


Trichoderma viridae


Aspergillus niger


Aspergillus flavus


Aspergillus flavus

4.5 IMPACT OF ECO-ENZYME ON THE BIOMETRICPARAMETERS OF GREEN GRAM

The result of germination percentage, root length, shoot length and vigour index of fenugreek seedlings grown with 0.5% of eco-enzyme (T_1) and tap water (T_2) on 7th daywas depicted in table 3. Plate 6 shows the growth of green gram on 7th day.

PLATE 6

Pot culture experiment

T_1 – 0.5% eco-enzyme

T_2 – Tap water (control)

Germination percentage and Vigour index

The seeds of green gram show highest percentage of germination (100%) in T_1 followed by T_2 (100%).

The shoot length and root length of fenugreek was maximum in T_1 (6.55cm and 7.35cm) followed by T_2 (4.7cm and 4.02cm) respectively (Plate 7).

The vigour index was maximum in T_1 (742) and minimum was observed in T_2 (474) (Table 3).

Table 3

Biometric parameters in 7 days old seedlings of green gram

Treatment	Growth percentage	Root length(cm)	Shoot length(cm)	Vigour index
T_1	100	7.35	6.55	742
T_2	100	4.7	4.02	474

(Values are the mean of triplicates)

PLATE 7

Shoot and root length of green gram grown using eco-enzyme

Seed germination is a single step process in the life cycle of a plant,which is very complex and influenced by many environmental factors (Ramagopal, 1998). Among the growth processes, seed germination and seedlings growth have been considered critical for raising successful agricultural crop. The processes of germination and growth of young seedlings are susceptible to toxic materials.

Eco-enzyme has a significant role in plant growth which might be due to the fact that it can convert ammonia into nitrate which acts as a fertilizer. It can also transform Carbon dioxide into Carbonatewhich helps in the nourishment of plants and protects the environment. It also produces antioxidants which reduce the ageing of cells.

Eco-enzymes also enhance the rate of photosynthesis which in turn makes the plants to get more nutrients and their roots can absorb more air from the atmosphere. Also the ozone emitted from by the eco-enzyme helps to grow faster and better. The diluted eco enzyme had an effective role when sprayed on a barren land, the plants started to grow well.

Hence it is very useful in agriculture and acts as a natural fertilizer to plants and makes the barren land fertile.

4.6 IMPACT OF ECO-ENZYME ON THE GROWTH OF *Catlacatla*

The results of the present study revealed that there was no mortality in the fishes grown in tap water and 0.5% eco-enzyme on 30th and 60th day respectively. The length and weight of the fishes were recorded to be 5.7% and 19% in control whereas in fishes grown in eco-enzyme there was an increase in the length and weight 13% and 38% on 30th day. As the exposure of fishes to the eco-enzyme was increased the length and weight were also increased gradually to 25% and 69% on 60th day respectively(Table 4). Plate 8 & 9 shows the experimental condition for the growth of fishes.

Table 4

Growth parameters of Catla catla treated with eco enzyme

Days of exposure	Weight gain%		Length gain%	
	Control	0.5%	Control	0.5%
30	19%	38%	5.7%	19%
60	44%	69%	14%	25%

Thus the result reveals that eco-enzyme can be given as food to the fishes which have the capacity to boost the immune system and also improve the quality of fish meat. It also replaces the drugs or additives which are provided to keep the animals in a healthy condition. (Wiswasta*et al*.,2012)

PLATE 8

Experimental set up for the study

T_1 – 0.5% eco-enzyme

T_2 – Tap water (control)

PLATE 9

Experimental fish – Catla catla

4.7 LARVICIDAL ACTIVITY

The eco-enzyme had a capacity to kill the mosquito larvae at 100%level in 20% and raw enzyme whereas the mortality was recorded to be 20%,50%and 60% in 5%,10% and 15% respectively. In control there was no mortality respectively. This might be due to the acidic nature of the eco-enzyme which might have penetrated into the larvae and caused mortality (Table 5). Plate 10 shows the larvicidal activity of eco-enzyme.

Thus to keep the environment free from mosquitoes eco-enzyme at a concentration of 20% or raw can be sprayed into stagnant water and can protect from mosquito borne diseases.

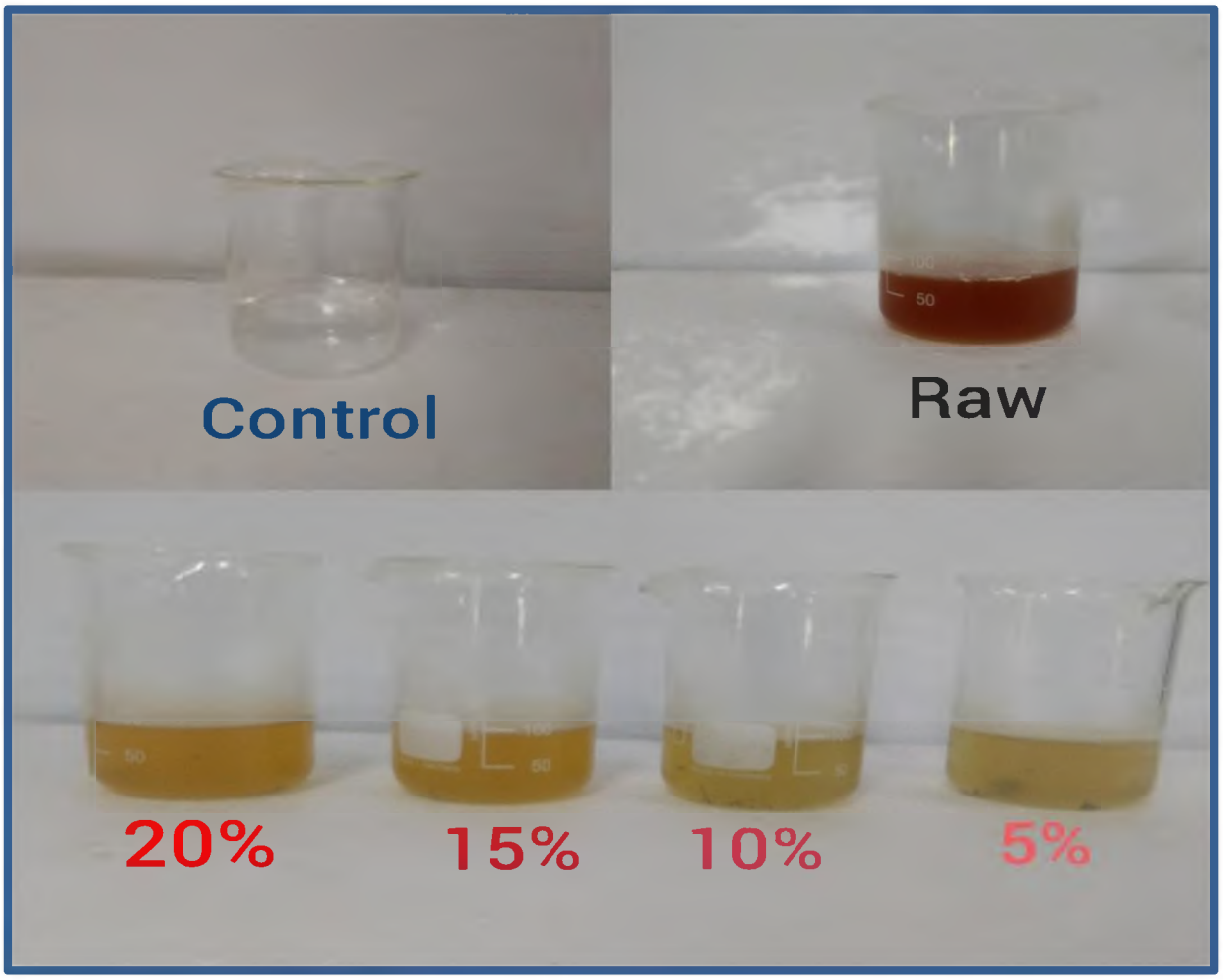
TABLE 5

Larvicidal activity of eco-enzyme against mosquito larvae

Treatment (%)	Percentage of mortality(%)
Control	0
5	20
10	50
15	60
20	100
100	100

PLATE 10

Larvicidal activity of eco enzyme



Control – tap water

Raw – 100% eco-enzyme

20% – 20ml eco-enzyme in 80ml of tap water

15% – 15ml eco-enzyme in 85ml of tap water

10% – 10ml eco-enzyme in 90ml of tap water

5% – 5ml eco-enzyme in 95ml of tap water

4.8 IMPACT OF ECO-ENZYME IN THE REMOVAL OF METHYL RED STAIN FROM FABRIC

The stained fabric was exposed to the raw eco-enzyme for a duration of 0-30minutes. The raw eco-enzyme had the ability to remove the stain from the fabric within 15mins which might be due to the presence of lipase and cellulase present in the eco-enzyme. This may catalytically act on the stain and may help on its removal (fig.3).

Thus the eco enzyme proves to be a bio-cleaner to remove dirt and stains from the fabric.

Fig. 3

5. SUMMARY AND CONCLUSION

In the present study the eco-enzyme produced from the decomposable organic waste were used to determine its applications in various fields.

The salient findings of the study are stated below:

- The eco enzyme produced by the fermentation was tested for its physical and chemical characters. The pH in the eco-enzyme revealed to be acidic in nature.
- The eco-enzyme was tested for presence of enzymes namely protease, lipase, maltase, invertase and amylase qualitatively. It was observed that the eco-enzyme possess maltase, invertase, protease and amylase respectively.
- The eco-enzyme was also tested for its antimicrobial activity against the selected bacterial (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes*) and fungal isolates (*Aspergillusniger, Aspergillusflavus,Rhizopussp,Alterneriasp, Trichodermaviridae*) using 0.5% eco-enzyme. The results confirm that the eco-enzyme has the capacity to inhibit the growth of microbes which was authenticated by the formation of zones.
- The eco-enzyme was tested to check its nature against growth parameters (germination percentage, shoot length, root length and vigour index) of green gram. It was observed that there was 100% germination in the seeds indicating it as a good fertilizer and not as a toxicant.
- The eco-enzyme was checked for its efficiency to use as a feed for the fish *Catla catla.* The fishes were exposed to 0.5% of eco-enzyme and tap water for 60 days. There was no mortality in fishes and the length and weight was increased on 60^{th} day when compared to 30^{th} day. It

revealed that eco-enzyme can be a good alternative supplement for fishes.

- Larvicidal potential of eco-enzyme was tested with different concentrations of enzymes (5%, 10%, 15%, 20% and raw eco-enzyme). It was observed that 100% mortality was observed in 20% and raweco-enzyme.
- The raw eco-enzyme was also checked as a stain remover against the dyed fabric for a period of 30mins. The eco-enzyme had the ability to remove the stain within 15mins indicating it to be bio-cleaner.
- Thus to conclude, the eco-enzyme proves to contribute in effective management of organic wastes and provide a clean eco-friendly environment without wastes and pollution.

6. REFERENCES

- Abdul-Baki, A.A. and Anderson, J.D., 1973. Vigor determination in soybean seed by multiple criteria 1. *Crop science*, *13*(6), pp.630-633.
- AmaleshSamanta, Pinaki Pal, AnurupMandal, ChandrimaSinha, AsifLalee, Mousumi Das, SudiptaKaity, DebmalyaMitr (2012). Estimation of Biosurfactant Activity of an Alkaline Protease Producing Bacteria Isolated from Municipal Solid Waste. Central European Journal of Experimental Biology, 1 (1):26-35.
- Andre L. Rodrigues, Angelica Cavalett, Andre O.S. Lima (2010). Enhancement of Escherichia coli cellulolytic activity by coproduction of beta-glucosidase and endoglucanase enzymes. Electronic Journal of Biotechnology,13(5):1-9.
- Arun, C. and Sivashanmugam, P., 2015. Identification and optimization of parameters for the semi-continuous production of garbage enzyme from pre-consumer organic waste by green RP-HPLC method. *Waste management*, *44*, pp.28-33.
- Arun, C. and Sivashanmugam, P., 2015. Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. *Process Safety and Environmental Protection*, *94*, pp.471-478.
- Arun, C. and Sivashanmugam, P., 2015. Solubilization of waste activated sludge using a garbage enzyme produced from different pre-consumer organic waste. *RSC Advances*, *5*(63), pp.51421-51427.
- Ashfaq,. A and Khatoon, A., 1970. Prevention of Environmental Degradation by means of solid waste management. *I Control Pollution*, *29*(1).
- Bae, S., and Makoto, S. Bacterial cellulose production by fed-batch fermentation in molasses medium. BiotechnolProg. Vol. 20, pp. 1366-1371, 2004.

- Beszedes, S., Szabó, G.G. and Géczi, G., 2012. Application of thermal and microwave pre-treatments for dairy wastewater sludge. *Annals of Faculty Engineering Hunedoara-International Journal of Engineering*, *10*(3), pp.231-235.
- BhavaniPrakash, 2011. How to make and use garbage enzyme. http://www.ecowalkthetalk.com (accessed 17.12.13).
- Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248_254.
- Carlos Regalado, ImmerVázquez-Obregón, Blanca Estela García-Almendárez, Jorge Domínguez-Domínguez, Araceli Aguilera-Barreyro, Aldo Amaro-Reyes (2010). Xylanolytic enzymes production by Aspergillusniger GS1 from solid-state fermentation on corn stover and their effect on ruminal digestibility. Electronic Journal of Biotechnology,14(2):1-11.
- Castrejón-Godínez, M.L., Sánchez-Salinas, E., Rodríguez, A. and Ortiz-Hernández, M.L., 2015. Analysis of solid waste management and greenhouse gas emissions in Mexico: A study case in the central region. *Journal of environmental protection*, *6*(02), p.146.
- ChamrajGokulMadhumithah, RaghavanKrithiga, Sridhar Sundaram, ChangamSheelaSasikumar, Soma Guhathakurta and KotturathuMammenCherian (2011). Utilization of Vegetable Wastes for Production of Protease by Solid State Fermentation Using Aspergillusniger. World Journal of Agricultural Sciences, 7 (5): 550-555.
- ChandrashekharUnakal,Radha I. Kallur, Basappa B. Kaliwal (2012). Production of αamylase using banana waste by Bacillus subtilis under solid state fermentation. European Journal of Experimental Biology, 2012, 2 (4):1044-1052.

- Csillakatalindezsi (2011). Optimization of invertase production by Yeast strains from the genus Saccharomyces. ActaUniversitatisCibiniensis Series E: Food technology, XV (2) : 11-18.
- Deusanilde J. Silva, Orlando J. Rojas, Martin A. Hubbe, Song W. Park (2011). Enzymatic Treatment as a Pre-Step to Remove Cellulose Films from Sensors. Macromol. Symp, 299/300:107–112.
- DuBois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. (1956). Colorimetric methodfor determination of sugars and related substances. Analytical Chemistry, 28(3),350–356.
- Emimol A, Ganga G, Parvathy R, Radhika G, Nair GM (2012). Screening of microbes producing extracellular hydrolytic enzyme from corporation waste dumping site and house hold waste for the enhancement of bioremediation methods. IOSR-JPBS. 2012;4:54–60.
- Eva Hostinova (2002). Amylolytic enzymes produced by the yeast Saccharomycopsisbuligera. Biologia, Bratislava, 57(11) : 247-251. .
- Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C., Angioni, A., Dessi, S., Marzouki, N. and Cabras, P., 2008. Comparative analysis of polyphenolic profiles and antioxidant and antimicrobial activities of tunisian pome fruit pulp and peel aqueous acetone extracts. *Journal of agricultural and food chemistry*, *56*(3), pp.1084-1090.
- Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C.I., Angioni, A., Dessi, S., Marzouki, N. and Cabras, P., 2007. Antimicrobial activity of Tunisian quince (Cydoniaoblonga Miller) pulp and peel polyphenolic extracts. *Journal of Agricultural and Food Chemistry*, *55*(3), pp.963-969.
- Gail Lorenz Miller (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar.Analytical Chemistry, 31 (3) ; 427-428.
- Gareth Evans, Ian Smith, Stephen Stagg , Howard Mason (2013). A survey of exposure to enzymes in cleaning solutions used to clean endoscopes. Health and safety Executive,113.

- Govindarajan, M. and Sivakumar, R., 2012. Adulticidal properties of Cardiospermumhalicacabum plant extracts against three important vector mosquitoes. *Eur Rev Med PharmacolSci*, *3*, pp.95-104.
- Gupta, S., Mohan, K., Prasad, R., Gupta, S. and Kansal, A., 1998. Solid waste management in India: options and opportunities. *Resources, conservation and recycling*, *24*(2), pp.137-154.
- Henderson, S.B., Beckerman, B., Jerrett, M. and Brauer, M., 2007. Application of land use regression to estimate long-term concentrations of traffic-related nitrogen oxides and fine particulate matter. *Environmental science & technology*, *41*(7), pp.2422-2428.*erica Journal*, *60*(3), pp.855-859.
- Howard R.L., Abotsi E., Jansen van Rensburg E.L., Howard S. (2003).Lignocellulose biotechnology: issues of bioconversionand enzyme production. African Journal of Biotechnology, 2 (12) : 602-619.
- Janarthanan .R.,P.Prabhakaran, P.M.Ayyasamy (2014). Bioremediation of vegetable wastes through biomanuring and enzyme production. Int.J.Curr.Microbiol.App.Sci ., 3(3): 89-100.
- Joeanoon, 2008. http://veg4planet.blogspot.in/2008/07/garbage-enzyme-anti-greenhouse-effect.html (accessed 17.10.12).
- Kanagaraj. J. (2009). Cleaner Leather Processing By Using Enzymes: A Review.Advanced Biotech,13-18.
- Kenthorai Raman Jegannathan, Per Henning Nielsen. (2013). Environmental assessment of enzyme use in industrial production a literature review. Journal of CleanerProduction, 42:228-40.
- Khan, M.A. and Ghouri, A.M., 2011. Environmental pollution: its effects on life and its remedies. *Researcher World: Journal of Arts, Science & Commerce*, *2*(2), pp.276-285.
- Kovendan, K., Murugan, K., Vincent, S. and Kamalakannan, S., 2011. Larvicidal efficacy of Jatrophacurcas and bacterial insecticide, Bacillus

thuringiensis, against lymphatic filarial vector, Culexquinquefasciatus Say (Diptera: Culicidae). *Parasitology research*, *109*(5), pp.1251-1257.

- Lou, X.F. and Nair, J., 2009. The impact of landfilling and composting on greenhouse gas emissions–a review. *Bioresource technology*, *100*(16), pp.3792-3798.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurements with Folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
- Mahmood, A.U., Greenman, J. and Scragg, A.H., 1998. Orange and potato peel extracts: Analysis and use as Bacillus substrates for the production of extracellular enzymes in continuous culture. *Enzyme and microbial technology*, *22*(2), pp.130-137.
- Mohareb, E.A., MacLean, H.L. and Kennedy, C.A., 2011. Greenhouse gas emissions from waste management—assessment of quantification methods. *Journal of the Air & Waste Management Association*, *61*(5), pp.480-493.
- Murugan, K., Kumar, P.M., Kovendan, K., Amerasan, D., Subrmaniam, J. and Hwang, J.S., 2012. Larvicidal, pupicidal, repellent and adulticidal activity of Citrus sinensis orange peel extract against Anopheles stephensi, Aedesaegypti and Culexquinquefasciatus (Diptera: Culicidae). *Parasitology research*, *111*(4), pp.1757-1769.
- Nayak .K.K., A. Tiwari, S.N.Malviya (2011). Production of taqpolymerase from E. Coli: a tremendous approach of Cloning and expression of Taqpolymerase-i gene in e. Coli. Advance research in pharmaceuticals and Biologicals,1(2):60-75.
- Nazim, F. and Meera, V., 2013. Treatment of synthetic greywater using 5% and 10% garbage enzyme solution. *Bonfring International Journal of Industrial Engineering and Management Science*, *3*(4), pp.111-117.

- Nguyen Hoang Loc, Do Van Giap, Hoang Tan Quang(2011). Production of NPRC10 protease by recombinant Escherichia coli through submerged culture in 40-L fermenter. Annals of Biological Research, 2 (6): 62-68.
- Olorunnisola Kola Saheed, Parveen Jamal, Mohamed Ismail Abdul Karim, ZahagirAlam, SuleymanAremuMuyibi (2013). Cellulolytic Fruits Wastes: A Potential Support for Enzyme Assisted Protein Production. Journal of Biological Sciences, 13: 379-385.
- Olsen, H.S. and Falholt, P., 1998. The role of enzymes in modern detergency. *Journal of Surfactants and Detergents*, *1*(4), pp.555-567.
- Omojasola, P. Folakemi, Jilani, Omowumi Priscilla, Ibiyemi, S.A. (2008). Cellulase Production by some Fungi Cultured on Pineapple Waste. Nature and Science, 6(2): 6479.
- Osuntoki AA, Ejide OR, Omonigbehin EA (2008) Antagonistic effects on Enteropathogenic and plasmid analysis of Lactobacilli isolated from fermented Dairy products. Biotechnology 7: 311-316.
- Panneerselvam, C. and Murugan, K., 2013. Adulticidal, repellent, and ovicidal properties of indigenous plant extracts against the malarial vector, Anopheles stephensi (Diptera: Culicidae). *Parasitology research*, *112*(2), pp.679-692.
- Parkar .S.G., Flint .S.H., Brooks.J.D (2004). Evaluation of the effect of cleaning regimes on biofilms of thermophilic bacilli on stainless steel. Journal of Applied Microbiology,96:110-116.
- Pote, D.H., Daniel, T.C., Moore, P.A., Nichols, D.J., Sharpley, A.N. and Edwards, D.R., 1996. Relating extractable soil phosphorus to phosphorus losses in runoff. *Soil Science Society of Am*
- Prakash, B., 2011. How to make and use garbage enzyme. *Retrieved*, *2*(09), p.2012.

- Prasad rao P.V.V., Satya CHV, Sri rami reddy.D (2014). Jack fruit waste: a potential substrate for pectinase production. Indian j.sci.res.,9 (1): 058-062
- Rahna. K. Rathnan ,Ambili. M (2011). Cellulase Enzyme Production by Streptomyces Sp Using Fruit Waste as Substrate. Australian Journal of Basic and Applied Sciences, 5(12): 1114-1118.
- Rasit, N. and Kuan, O.C., 2018, April. Investigation on the Influence of Bio-catalytic Enzyme Produced from Fruit and Vegetable Waste on Palm Oil Mill Effluent. In *IOP Conference Series: Earth and Environmental Science* (Vol. 140, No. 1, p. 012015). IOP Publishing.
- Rasit, N. and Mohammad, F.S., 2018. Production and characterization of bio catalytic enzyme produced from fermentation of fruit and vegetable wastes and its influence on aquaculture sludge. *Matter: International Journal of Science and Technology*, *4*(2).
- Renge V.C., S. V. Khedkar, Nikita R. Nandurkar (2012). Enzyme Synthesis By fermentation Method : A review. Sci. Revs. Chem. Commun.: 2(4), 585-590.
- Saravanan P., R. Muthuvelayudham, T. Viruthagiri (2012). Enhanced Production of Cellulase from Pineapple Waste by Response Surface Methodology. Journal of Engineering,
- SedighehJavadpour, DariushNorouzian, AzimAkbarzadeh, SaeedMirdamadi, Behrokhfarahmand (2002). Isolation of a Penicillin Acylase Producing E.coli and Kinetic
- Selvakumar, P. and Sivashanmugam, P., 2018. Multi-hydrolytic biocatalyst from organic solid waste and its application in municipal waste activated sludge pre-treatment towards energy recovery. *Process Safety and Environmental Protection*, *117*, pp.1-10.

- Srilatha, H. R., Krishna, N., Sudhakar, B. K., K. Madhukara, 1995. Fungal pretreatment of orange processing waste by solid state fermentation for improved production of methane. Process Biochem ., 30:327-31.
- Syahirah, M.F. and Nazaitulshila, R., 2018. The Utilization of Pineapples Waste Enzyme for the Improvement of Hydrolysis Solubility in Aquaculture Sludge. *Journal of Energy and Safety Technology (JEST)*, *1*(2-2).
- Tang, F.E. and Tong, C.W., 2011. A study of the garbage enzyme's effects in domestic wastewater. *International Journal of Environment, Chemical, Ecological, Geological and Geophysical Engineering*, *5*, pp.887-892.
- TengkuNorsalwani T.L., N. A. NikNorulaini (2012). Utilization of Lignocellulosic Wastes as a Carbon Source for the Production of Bacterial Cellulases under Solid State Fermentation. International Journal of Environmental Science and Development, 3(2):136140.
- Toca-Herrera J.L, J. F. Osma, S. Rodríguez Couto (2007). Potential of solid-state fermentation for laccase production. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 391- 400.
- Tokpohozin, S., Fall, J., Abdoulaye, L.o.u.m., Sagne, M. and Diouf, M., 2015. International Journal of Advanced Research in Biological Sciences. *Int. J. Adv. Res. Biol. Sci*, *2*(11), pp.143-154.
- Triassi, M., Alfano, R., Illario, M., Nardone, A., Caporale, O. and Montuori, P., 2015. Environmental pollution from illegal waste disposal and health effects: A review on the "Triangle of Death". *International Journal of Environmental research and public health*, *12*(2), pp.1216-1236.
- Viturtia, A., Alvarez, J. M., Cecchi, F and G. Fazzini, 1989. Two-phase anaerobic digestion of a mixture of fruit and vegetable wastes. Biol Wastes., 29:189-99.

- Weber, R., Watson, A., Forter, M. and Oliaei, F., 2011. Persistent organic pollutants and landfills-a review of past experiences and future challenges. *Waste Management & Research*, *29*(1), pp.107-121.