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PRODUCTION, EXTRACTION AND USES OF ECO-ENZYME USING CITRUS FRUIT WASTE: WEALTH FROM WASTE

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Abstract–Eco-enzymes is a complex dark brown colour solution produced by fermentation of fruits waste. It has strong sweet and sour fermented scent due to citrus fruit peels. Eco enzyme produced using fruit peels, water and brown sugar in a ratio 3:10:1. After incubation the filtrate was obtained, we found Flavonoids, Alkaloids, Quinones, Saponins as presence of different metabolites. Its IR spectra showed presence of -OH, COOH group. Also, Amylase, protease and lipase were found in the filtrate. We found applicability as floor cleaning, utensils, gardening, etc. the novel approach for recycle and reuse natural waste would help to reduce fruit waste, it is eco-friendly, economical with multipurpose application.

INTRODUCTION

Eco-Enzyme is a kind of organic compound. It is a complex solution produced by fermentation of fresh kitchen waste such as vegetable and fruit peels. It is type of homebrew vinegar, reduced from alcohol by fermentation of kitchen waste as substrate with sugar (Xia Li, hang Wang, 2013). Eco-enzyme can be prepared generally from citrus fruit peels or kitchen waste. Citrus fruit peels are used due to their distinct properties such as fragrance and sharp flavour, source of vitamin C and also rich in medicinal properties along with high acidity value. The sugar which is added is utilized by microbes; due to their metabolism ozone that is derived may kill the bacteria (Pinang)

Eco-enzyme acts as an anti-fungal, anti-bacterial and insecticidal agent. It may also use as cleansing agent. In the present study we work on production of Eco-enzyme from citrus fruit peels and their different applications.

MATERIALS AND METHODS

Citrus peels were collected from fruit vendors, local fruit juice shop, brown sugar was purchased from local grocery (Maharashtra provisions), water used was normal tap water, all the media and chemical used were purchased from Hi-Media (Mumbai), and reagents used were of AR grade.

Production of Eco-Enzyme

The citrus fruit peels (sweet lime, orange, and lemon) were collected washed thoroughly under running tap water and were chopped into small pieces. Then 500 g of brown sugar was added to 5lts of water and stirred until sugar dissolved completely, to it chopped 1.5kg fruit peels were added. The solution was incubated for 3 months in airtight plastic container. Initially the mixture was stirred daily using glass or wooden rod to release the gas formed by fermentation. Further in second month the solution was mixed once in two weeks followed by once in middle of third month.

Enzyme assay

The crude sample was analysed for various enzyme activities including protease, amylase and lipase.

Enzyme assay Protease

Eco-Enzyme crude sample was serially diluted 10^{-1} to 10^{-4} and spread on Skimmed milk agar plates (skim milk powder-28g/L, yeast extract-2.5g/L, dextrose-1g/L, tryptone-5g/L, agar-15 g/L) (with pH 7.0) and incubated at 37 °C for 48 hrs. the positive isolates were further screened for better production of enzyme by assaying the protease activity in liquid culture using casein as substrate at 37 °C

(Hanan S. Alnahdi, 2012).

Enzyme assay Amylase

Eco-Enzyme crude sample was serially diluted 10^{-1} to 10^{-4} and 0.1 mL sample was spread on each starch nutrient agar plates. After incubation for 1-2 days at 37 °C plate was flooded with Gram's iodine and observed for zone of hydrolysis (Ann kipps and P. H. whitehead).

Enzyme assay Lipases

Eco-Enzyme crude sample was serially diluted 10^{-1} to 10^{-4} and spread plated 0.1 mL on Lipid agar (Glucose-1g, tributyrin-4 mL, NaCl-0.5g, NH_4Cl-4.5g, $MgSO_4$–0.5g, D/W-100 mL, agar-agar-2.5g/100mL) and incubated for 37 °C for 24 hrs. The plates were observed for zone of clearance (Suseela Lanka and J. Naveena Lavanya Latha, 2015).

Identification of metabolites

Tests were carried out to confirm the presence of flavonoids, alkaloids, quinones, cardenolides, and saponins.

Alkaline regent test

The 2 mL of sample was treated with few drops of 20% NaOH solution and observed for change in intense yellow colour to colourless solution on addition of dilute HCL.

Wanger's reagent

The 2 mL of Sample was treated with 3-5 drops of Wanger's reagent (1.27g of iodine and 2g of KI in 100 mL of water) and observed for formation of reddish/brown precipitate.

Keller test

5mL of sample was treated with 2 mL of glacial acetic acid in a test tube and few drops of 5% $FeCl_3$ solution was added to it. This was carefully under layered with 1 mL concentrated H_2SO_4, awaited to form brown ring at interface (which is due to deoxy sugars) characteristics of cardenolides.

Ferric chloride test

A 2 mL of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue colour, which shows absence of phenols.

Foam test

2 mL of sample was added in 6 mL of water and was shaken vigorously and observed for formation of foam.

Test for Quinones

2mL of sample was treated with concentrated HCl and observed for formation of yellow precipitate.

Quantification of Acetic acid

The 10 mL of sample was titrated against the titrant 1M NaOH. The few drops of phenolphthalein indicator was added and observed for the formation of pink colour.

Antimicrobial activity

The nutrient agar plates were spread with different microbial suspension (*E.coli, Pseudomonas ssp., Bacillus* spp.) and 1 mL of sample was inoculated using well diffusion method. These plates were incubated for 48 hrs at 32 °C and observed for zone of inhibition.

Effect on plantlet growth

Two containers of soil were taken and labelled as with and without Eco-enzyme, each container added 25 seeds of wheat and 5 mL diluted Eco-enzyme and container kept for plantlet growth.

RESULTS AND DISCUSSION

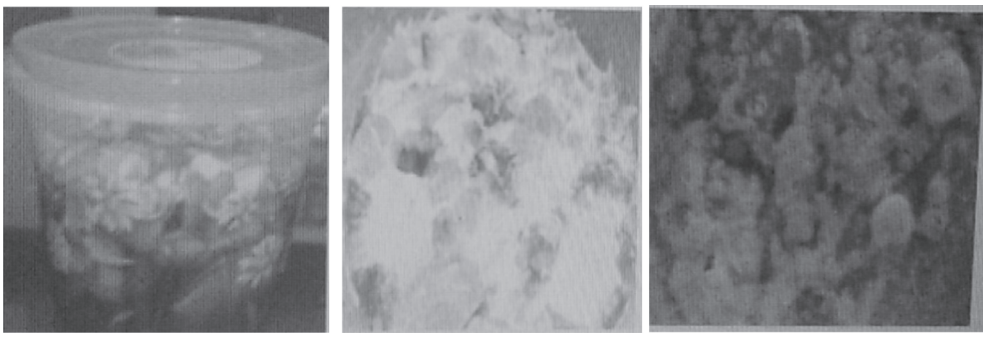
Eco-enzyme solution was produced by using citrus fruit peels. Mostly in this experiment we used citrus fruit peels as in Nanded region they are cultivated on large scale. These citrus fruit peels were collected from various shops of Nanded. The collected peels and juice shop fruit waste survey is follows (Table 1)

Table 1. The survey result of juice shops for peels of citrus fruit (approx. in kg).

Fruit	No. of shops	Fruit waste (kg)	Waste/day
Sweet lime	25	7	175
Oranges	25	6	150
Pineapple	25	4	110
All fruits	25	56	250

After collection of these fruit peels, these were sorted and cleaned and were further used as substrate for Eco-enzyme production. Around 1.5 kg of citrus fruit peels along with brown sugar and water were kept in air tight container and was incubated for 1st cycle of fermentation (Fig 1-a, b, c).

After fermentation of three months, crude



(a) (b) (c)

Fig. 1. Formation of Eco-Enzyme: (a) mixture in air tight container, (b) white layer formed after two weeks, (c) brown crude filtrate after 3 months

solution was filtered and analysed further for presence of different metabolites, enzymes. Based on these tests different application was proposed.

The formation of clear zone around the colonies confirms the production of alkaline protease (Fig. 2a). The zone of hydrolysis and clearance indicates the presence of amylase and lipase enzyme (Fig 2b and 2c).

Further the crude solution was analysed for metabolites presence the intense yellow colour change to colourless solution on addition of dilute HCl acid, indicating Flavonoids presence (Fig 3a). Formation of yellow precipitate was observed, indicating presence of Quinone's (Fig 3b). On shaking vigorously foam formation was observed, indicating Saponins presence (Fig 3c). There was formation of reddish-brown precipitate, indicating Alkaloids presence (Fig 3d). A brown ring at interface shows the presence of deoxy sugar characteristics of cardenolides (Fig 3e).

Antimicrobial activity

The antimicrobial activity was shown against *Pseudomas spp.*, *E.coli*, *Bacillus spp.* Highest antimicrobial activity was shown against *Bacillus spp.* with 18 mm zone of inhibition (Fig 4c). Whereas, 11 mm and 5mm zone of inhibition was shown against *Pseudomas spp.*, *E.coli*, respectively (Fig 4a, 4b)

Quantification of Acetic acid:

The 10 mL Eco-Enzyme contains 0.084 mL of acetic acid which is around 42.25 mL in 500 mL Eco-enzyme thus the pH of Eco-Enzyme is acidic it can be brought to basic upon dilution (Fig 5).

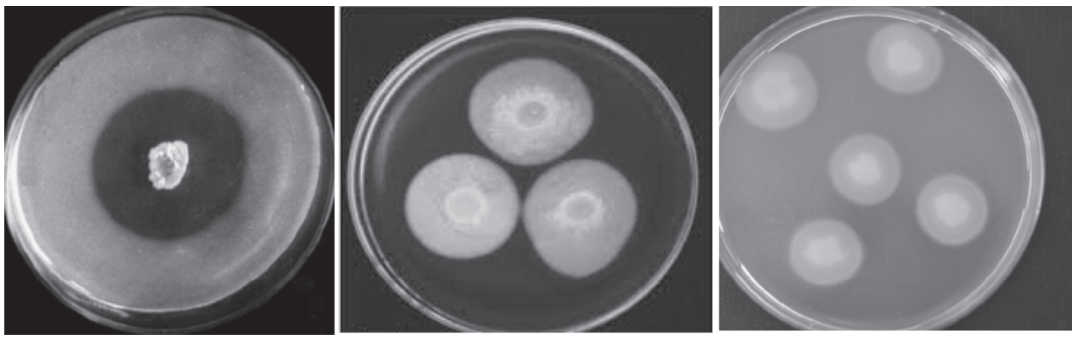
IR Spectra

The crude sample was filtered from membrane filter so that no microbes will enter in liquid. The IR spectra of filtered liquid was performed that represents the presence of -OH and -COOH functional groups (band of 3303.83/cm and the band of 1637.45/cm were observed respectively (Fig 6).

Applications of Eco-enzyme

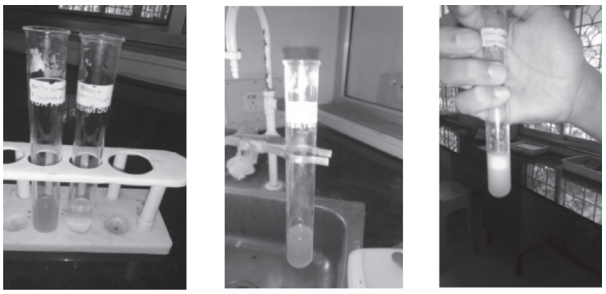
1) Enhanced plant Growth

It was observed that the generation time of seedling was 6 days with Eco-Enzyme while seedlings took 9 days to grow without Eco-Enzyme. Also, seedling



(a) (b) (c)

Fig. 2. Enzyme assay (a) Protease activity, (b) Amylase activity, (c) Lipase activity



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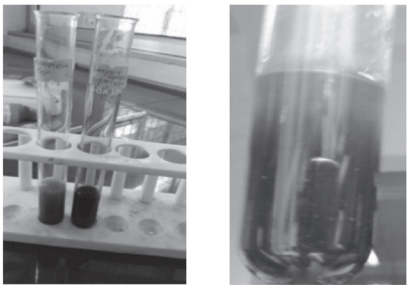
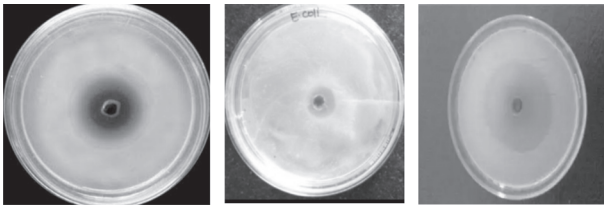


(d) (e)

Fig. 3. Metabolites: (a) Flavonoids, (b) Quinone's, (c) Saponins, (d) Alkaloids, (e) Cardio glycosides



(a) (b) (c)

Fig. 4. Antimicrobial activity against (a) *Pseudomonas spp*., (b) *E.coli*, (c) *Bacillus spp.*

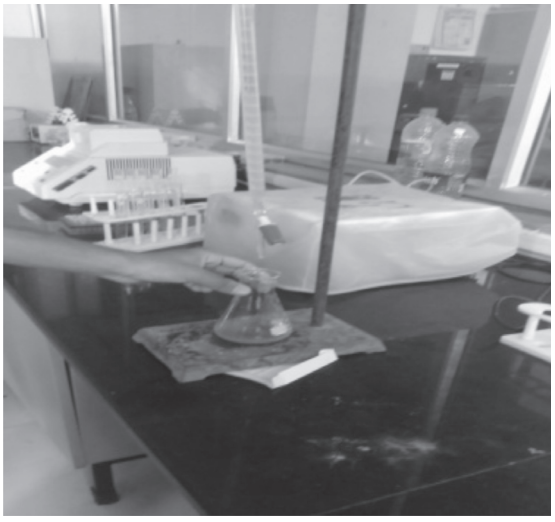


Fig. 5. Formation of light pink colour indicating acetic acid presence.

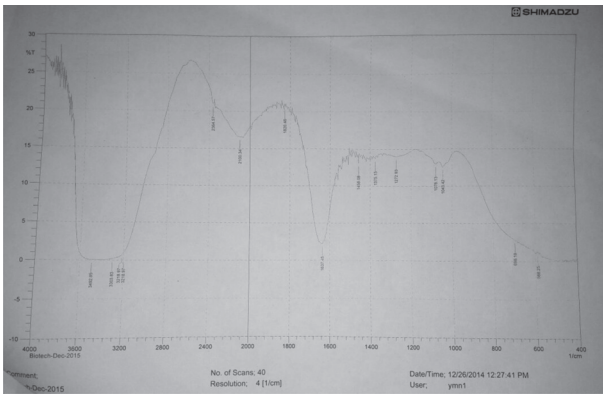


Fig. 6. FTIR spectra of crude filtrate

Table 2. The number of components used in production of Eco-Enzyme

Sr. No.	Components	Cost
1	Brown sugar	50/-
2	Water	0/-
3	Fruit peels	0/-
4	Container	50/-

Table 3. Comparison of Eco-enzyme cost with other available commercial products.

Sr. No.	Commercial products	Market cost/200mL	Eco-Enzyme/ 200 mL
1	Lizol	27/-	10/-
2	Dettol	35/-	10/-
3	Lifeboy	30/-	10/-
4	Vim	18.5/-	10/-

vigour was more with Eco-enzyme than seedling without Eco-enzyme. (Fig 6-a, b, c).

In household application

Eco-Enzyme due to it acidic nature is used in Cleaning of utensils, floor cleaning. Also due to it smell it repels the mosquitos.

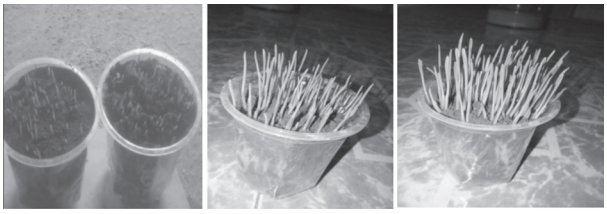
The cost efficiency of Eco-Enzyme

For production of Eco-enzyme citrus fruit peels and other components were used which are available at very cheaper cost (Table 1 and 2).

Whereas, when Eco-enzyme production cost was compared to other commercial products, it was found very cost effective (Table 3).

Eco-Enzyme comparison with commercial cleaning agents

Due to the presence of all-natural raw material



(a) (b) (c)

Fig. 7. (a) Seedling, (b) Seedling + water, (c) Seedling + water + Eco-Enzyme

present in the production it doesn't cause any hazardous or ill effect to environment while the chemical agents get accumulated in the nature and degrade the land over there and also may affect the water bodies over there. It also does not have any toxic effects over to human while few chemical agents do have ill effects. Due to the presence of natural microbes it activates the soil biology and helps in enhancement of plant growth and yield which has vice versa effect by chemical agents. Also, it has efficient commercial cleaning properties, it repels pest like mosquito. It also cheaper in rate, harmless and natural product (Table 4).

(a) (b)

Fig. 9. Application based on pest repellent and cleaning shown by (a) killed mosquito due to Eco-Enzyme and (b) bathroom tiles before and after use of Eco-Enzyme

The dilution amount required for particular application of Eco-Enzyme

As the Eco-Enzyme produced is in acidic nature it has to be diluted before the use for different purposes because the acidic nature may spoil the texture of things on which it is used. The acidic nature can be used for cleaning the floor, toilets, rust patches. But while cleaning the utensils, pets' body, it needs to be diluted as the surface may rust and cause irritation to pets. Further the plants also require the diluted form as many plants needs slight alkaline or neutral pH, also if soil is too much alkaline acidic nature may use to retain the natural pH of soil that is required (Table 5).

Ecological Significance

The produced Eco-enzyme is a multi usage product of kitchen waste which has ecological significant and is eco-friendly.

CONCLUSION

A critical need of the present day for reliable and

Table 4. Comparison of Eco-Enzyme with other chemical agents with respect to different chemical properties.

Sr. No.	Properties	Eco-Enzymes	Chemical agents
1	Production	From natural components	From chemical components
2	Nature	Acidic to alkaline	Most are acidic
3	Nature towards environment	Eco-friendly	Non eco-friendly
4	Cost	cheaper	Costly
5	Degradation	Degradable	Takes much time to degrade
6	Soil biology	Always activates it	Most of the time deactivates soil bilogy
7	Fumes	No production of fumes	Fumes are produced which are harmul
8	Toxic effects	No toxic effects on human	It fumes or other chemical component may be harmful to human

Table 5. Dilution rate for different usage

Sr. No.	Dilution rate	Usage	Application
1	Concentrated enzyme	Toilets, garden pond, water tank	Pour and clean/flush 2-3 times a week
2	10-15 times	Kitchen sink, black mould, pet house, stove, bathroom tiles	Spray occasionally
3	500 times	Drain, pet	Spray occasionally
4	100-1000 times	Purifying air, deodour, pest control	Spray frequently
5	More than 1000 times	Seedling and fertilizer	Spray occasionally

eco-friendly is fulfilled by Eco-Enzyme. Here we had reported the DIY procedure and low drift approach with loads of use for diminishing nourishment waste and basic and noteworthy strides towards lessening in greenhouse gases. In the present review endeavour was made to explore distinctive uses of Eco-Enzyme by estimation of various chemical compounds in it.

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