

The biodegradation behavior of an eco-characteristic Bio-formulated polymer nanosheets and eco-efficacy assessment for promoting growth of Eggplant (*Solanum melongena* L.)

T. Ruby Nirmala Mary

Anna University Chennai

Jayavel Ramasamy (✉ rjvel@annauniv.edu)

Anna University Chennai <https://orcid.org/0000-0001-6270-1484>

Research Article

Keywords: Bio-formulated polymer nanosheets, Cashew Nut Shell Liquid, Biodegradation, Migration Test, Eggplant (*Solanum melongena* L.)

Posted Date: March 8th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1376810/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The introduction of Cashew Nut Shell Liquid (CNSL), iron oxide nanoparticles, Plant extracts from *Mentha spicata* L., *Vitex negundo* L., and *Eucalyptus globulus* M., in chitosan biopolymer matrix has created a significant contribution for the development of Bio-formulated polymer nanosheets. The migration test performed for the formulated Bio-nanosheets have satisfactory results with very less amount of residue get migrated that revealed a suitable material for packaging application. The developed bio-formulated polymer nanosheets achieved an excellent biodegradation activity of about 80-93% through the acceleration of soil microbial flora. The SEM and EDAX analysis confirmed the surface degradability variation observed for 5 weeks. The present study is focused on the application of biodegraded soil for promoting the Eggplant growth (*Solanum melongena* L.) and its eco-efficacy evaluated by enhancing the development of an eco-characteristic active bio-nanomaterials which resulted a positive impact in packaging and agricultural sectors.

Highlights

- The developed Bio-formulated polymer nanosheets containing the natural plant extracts (*Mentha spicata* L., *Vitex negundo* L., *Eucalyptus globulus* M.) along with incorporation of CNSL natural phenolic binding resin, iron oxide nanoparticles and chitosan biopolymer.
- The lesser migration of residues from the developed material has revealed its promising packaging application.
- The Bio-formulated polymer nanosheets possesses an excellent eco-characteristic with natural biodegradation rate and the Eco-efficacy of the tested biodegraded soil which promotes the growth of nutritional eggplant (*Solanum melongena* L.) growth with good yield of brinjals.

1. Introduction

The growing challenge is concerned about the development of the food packaging material with less toxicity to the environment as well as marketing demand for the consumers satisfaction. The food contact materials used in the packaging industry can create a negative impact as a source of food contamination by chemical usage for the criteria of the production requirements. The essential step is to concentrate on the fabrication of the packaging material based on the chemical composition and the migration level of the compounds for consideration which is related to the human health and safety [1]. The demand of alternatives from bio sources for the production of an eco-friendly biomaterials is needed in the packaging industry. The researchers and the manufacturing industries are interested to focus on the future packages with tremendous use of the biomaterials for accelerating towards high -value products [2].

The incorporation of nanomaterials in the polymer matrix which enhances the functional properties such as antimicrobial, barrier properties and biodegradability nature as a sustainable food packaging material. The hydrophilic nature of chitosan biopolymer has proven its bio-functionality, biocompatibility, non-

toxicity and its biodegradability of breakdown into monomers offers an effective utilization with replacement of plastic material in food industry [3-4]. The biodegradable chitosan –based flexible films are used in food packaging application due to its antimicrobial characteristics [5]. The additional positive aspects of chitosan-based materials with bio adhesiveness and pH sensitiveness for future development in Active Intelligent packaging [6]. The nanocomposite-based graphene incorporated into the starch/chitosan matrix which reflects the advantage of being used in active packaging [7]. The application of biodegradable coating materials for the preservation of the fruit quality along with increase in the potentialities of agro-industrial bio economic benefits from renewable sources [8]. The improvement in longevity of the freshness in sea foods by developing an intelligent nanocomposite food packaging material [9]. The Food and Drug Administration (FDA) has accepted that the chitosan as a food ingredient due to its wide application for enhancing the shelf-life food products [10].

The blending of the plant extracts from natural sources which reduces the cost effectiveness and the biodegradability behavior can be improved with its application in biopolymer matrix. The biodegradation studies are usually done in soil environment. The significant change is caused by the microbial flora which is abundantly found in the soil [11, 12] and induces the enzymatic reaction which results in the production of the biomass. The biodegradation is not only influencing the environmental factors such as microbial growth, chemical composition of biopolymer and also the percentage of crystallinity [13].

The biological sources are converted into the nutrients through the compositing process that leads to the best solution of the solid waste management by inducing the material recycle process naturally [14]. The biodegradable polymers are an innovative promising material in the food packaging industry by converting it into the organic matter. The conversion of the organic matter into the natural by-products such as fertilizer, nutrients and energy by permitting back into the soil as a beneficial usage in the agricultural sector [15]. The characteristic nature of the chitosan-based material has been assessed with agronomic efficacy advantages in the plant production, protection from pests [16] and plant growth sensitivity [17]. The *Solanum melongena* L. (Eggplant) is grown with edible fruit bearing violet colored vegetable named as 'Brinjal' with impressive nutritional benefits containing vitamins, fiber, potassium, iron content, antioxidant and natural laxative. The eggplant growth is induced by foliar spraying of oligo-chitosan application which results in positive manner for promoting plant growth, yield and biochemical parameters [18].

The present study is aimed to develop a biodegradable Bio-formulated polymer nanosheets as a packaging material to overcome the persistent use of chemical toxicity and assessed the eco-efficacy of the biodegraded soil for promoting the eggplant (*Solanum melongena* L.) growth. The biodegraded Bio-formulated polymer nanosheets were converted into value added nutrients for soil enrichment with improvement in Eggplant growth and nutritional yield analysis for focusing towards the environmental benefits.

2. Materials And Methods

2.1 Materials:

Chitosan (Degree of Deacetylation 90%) used in the experiment along with some reagents Ferric nitrate nonahydrate, Ammonia Solution and Acetic acid (Glacial) were obtained from Sigma-Aldrich, India. Natural sources such as CNSL (Cashew nut shell liquid) was bought from Sathya Cashew Industry, Chennai. The plant leaves (*Mentha spicata* L., *Vitex negundo* L., and *Eucalyptus globulus* M.) were purchased from the local market, Tambaram, Chennai. The seeds of the COBH2(2009) brinjal variety were collected from Tamilnadu Agricultural university, Coimbatore, India. Soil Specimen test for biodegradability and Egg plant growth were done in delta region of Thanjavur district, Tamilnadu, India.

2.2. Development of the Bio-formulated polymer nanosheets:

The formulated Bio- polymer nanosheets were prepared by using solution casting method. In this method, Chitosan (2%) was dissolved in the acetic acid solution. The 0.1 ml natural binder CNSL and 0.45% w/v iron oxide nanoparticles were mixed with the chitosan solution. The concentration of 5% w/v plant extracts from the leaves of *Mentha spicata* L., *Vitex negundo* L., and *Eucalyptus globulus* M., were taken to obtain a Bio-formulated polymer nanosheets. The prepared solution was dried by casting on the plate at normal room temperature for 48 hrs and then analyzed for further use.

2.3. Characterization of the Bio-formulated polymer nanosheets:

2.3.1.SEM Morphology:

The surface morphology of the prepared Bio-formulated polymer nanosheets were imaged with Vega 3Tescan at 30 KV acceleration voltage with magnification 1000X to 5000X.

2.4. Overall migration test:

This method was used to determine the overall migration of constituents of the prepared Bio-formulated polymer nanosheets by the following standard method IS-9845. The test samples were immersed in the simulant solution of n-heptane and removed after the incubation period. Finally, the simulant was evaporated to dryness. The overall migration of simulant expressed in mg/dm² by the following Eq (1) and weighed using analytical balance.

$$\text{Amount of Extractives (Ex)} = \frac{\text{mass ratio of the residual simulant(m)}}{\text{sample after the residue}} \quad (1)$$

2.5. Investigation of Biodegradability Assessment using soil test:

The biodegradation of the prepared Bio-formulated polymer nanosheets were evaluated by using Soil degradation studies method.

2.5.1. Soil degradation studies:

The Test samples size (2x2cm²) and its initial weight were measured by using analytical balance. The prepared Bio-formulated polymer nanosheets were buried into the soil under aseptic conditions. The soil along with samples were exposed to all type of weather conditions (temperature, sunlight and humidity). The burial test period was about 5 weeks and intended to biodegrade directly into soil. For maintaining the natural wetness environment of degradation, the microbial flora was emanated by sprinkling of 10ml distilled water and it resulted in the enhancement of decomposition of the organic matter. The nutrients present in test samples containing carbon, nitrogen sources were utilized by microbes for its growth and activation which helps in biodegradation process.

2.5.2. Weight loss:

The Bio-formulated polymer nanosheets were taken from the degraded soil after 5 weeks. The removal of soil adherence from the test samples were washed with distilled water and dried under oven at 60 °C for 4 hrs. The readings of the test sample variation in size and weight were measured after reached its constant weight. The test samples were weighed before and after degradation for determination of the weight loss% by using the following eq (2)

$$WL \% = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (2)$$

2.5.3 SEM Imaging with EDAX Analysis:

The SEM analysis was used for examining the surface changes in the morphology of the prepared Bio-formulated polymer nanosheets. The images were analyzed by comparing the test samples before and after soil degradation for 5 weeks. The SEM Analysis was performed under the magnification of 5000X using Hitachi S-3400N model and operated at accelerated voltage of 5000 volt. The elemental composition of the developed Bio-formulated polymer nanosheets before and after soil degradation were studied by energy dispersive analysis of X-rays (EDAX).

2.6. Determination of microbial flora activity in the degraded soil:

The soil quantity of about 1kg was used for the biodegradation of the Bio-formulated polymer nanosheets. The biodegraded soil was assessed for the determination of the microbial flora such as yeast, molds and bacterial species (*Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*) by using microbiological analysis based on standard test method. After this test method, the soil was determined and identified with the presence of the microbial colonies active or not.

2.7. Environmental Impact Assessment:

The impact of the tested biodegraded soil was assessed in the Eggplant (*Solanum melongena* L.) for its evaluation of the viability seedling growth, plant growth parameters, nutrient quality and yield analysis. The seeds were sowed in the tested soil containing the composition of biodegraded Bio-formulated polymer nanosheets. After two weeks, the visible sprouts were protruding from the soil and monitored the

growth stage of eggplant. The adult eggplant with purple-colored brinjal were harvested for analysis of the nutrient content after it reached mature stage within 150 days.

The following Eq (3), Eq (4), Eq (5) for determination of germination rate and moisture content of the sprout growth

$$\text{Germination \%} = \frac{\text{No.of seeds germinated}}{\text{Total no .of seeds}} \times 100 \quad (3)$$

$$\text{MSD} = \frac{\text{Total no.of seeds germinated}}{\text{Total no .of days}} \quad (4)$$

$$\text{Moisture content \%} = \frac{\text{Fresh weight- Dry weight}}{\text{Fresh weight}} \times 100 \quad (5)$$

$$\text{Percentage of leaf dry matter \%} = \frac{\text{Leaf Dry weight}}{\text{Fresh weight}} \times 100 \quad (6)$$

3. Results And Discussion

3.1 Morphological features of the developed Bio-formulated polymer nanosheets

The prepared Bio-formulated polymer nanosheets exhibits a smooth, glossy and compact surface with fine iron oxide nanoparticles (~ 100 nm) dispersion are presented in the Figure 1 (a, b). The surface morphological studies of the prepared Bio-formulated polymer nanosheets are examined by SEM analysis. From Figure 1(d) the prepared Bio-formulated polymer nanosheets shows a good dispersion on CTS matrix. The blending of CNSL, Iron oxide nanoparticles and plant extracts are creating a complete surface modification.

3.2. Assessment of the overall migration of components from developed Bio-formulated polymer nanosheets

The Bio- formulated polymer nanosheets is determined for the overall migration into n-heptane stimulant according to the defined standard method IS-9845. The overall migration values result for the prepared Bio- formulated polymer Nano sheets (mgdm^{-2}) are summarized in Table 1 as CTS (1.4 ± 1), CTS- CNSL (3.77 ± 1), CTS-CNSL-Iron oxide Np (25.4 ± 1), CTS-CNSL-Iron oxide Np-P. E 1 (26.85 ± 1), CTS-CNSL-Iron oxide Np-P. E 2 (46.4 ± 1) and CTS-CNSL-Iron oxide Np-P. E 3 (50.9 ± 1) which represents that some components get migrated in residue form. The CNSL act a natural plasticizer which is creating a structural changes by enhancing the crystallinity and the overall migration value shows a lesser migration value in the range of $3.77 \pm 1 \text{ mg dm}^{-2}$.The value is increased after plant extract blending with prepared bio-formulated polymer nanosheets which are observed in Figure 2. Therefore, the use of CNSL as a natural binder which unalters the visual change and it results with reduction for the release of residues. This property plays an important role in primary food packaging application due to the interaction of packaging material with food surface contact and influences the less toxicity [19]

3.3 Examination of Biodegradability studies for the Bio-formulated polymer nanosheets

The biodegradability of the prepared bio-formulated polymer nanosheets are examined in soil for investigation of the natural flora which enhances the biodegradation process. Figure 3 represents the observation mechanism of the biodegradation process by using soil microbial flora for breaking the prepared Bio- formulated polymer nanosheets into constituent molecules. The important factor which plays a major role for biochemical transformation is due to microbial uptake of nutrients from the prepared sample as a source for enrichment of its growth. Figure 3(a) it is observed that the initial stage of degradation occurs, after the microbial interaction on the surface of the Bio- formulated polymer nanosheets. Figure 3 (b) explains the fore most stage of up taking nutrients of carbon from Bio- formulated nanosheets. The utilization results in the development of a microbial growth which leads to the breakdown of the prepared material [20]. The fragments of the biodegraded prepared Bio-formulated polymer nanosheets are clearly visible in the Figure 3(c) which enables the cleavage process towards the final step of conversion into monomers.

3.3.1. Weight loss percentage for the prepared Bio-formulated polymer nanosheets:

The tested samples are removed from the soil every week for the determination of the variation in weight mass after biodegradation process. The degraded bio-formulated polymer nanosheets are observed for the calculation of the weight loss percentage up to 5 weeks and records the mass loss % in the Table 2. The weight loss percentage is becoming more intense after 3 weeks than after 1 week but the higher percentage of mass loss registers for all the prepared samples about 80 to 93%. From Figure 3(d), the degree of biodegradation appears steady increase in weight loss % depending on burial time period. Comparing the results, the bio-formulated polymer nanosheets most evidently seems that the prepared material favors the biodegradation behavior. The biopolymer chitosan-based plant extract blends exhibit hydrophilic nature which could enhance the soil microbial community's growth results in faster degradation rate. The addition of natural resin CNSL and Iron oxide nanoparticles is getting slow difficult for hydrolysis due to its high crystallinity forms a stronger bonding (Covalent bond) with CTS matrix which resist the microorganism growth on the surface of the material results the controlled biodegradation rate at earlier up to 3 weeks.

3.4. Biodegradability Assessment of the Bio-formulated nanosheets in the soil environment

3.4.1. SEM Imaging with morphological observation of the biodegraded Bio-formulated polymer nanosheets:

The morphological surface change of roughness is observed in the prepared Bio-formulated polymer nanosheets after biodegradation up to 5 weeks and are presented in Figure 4(a) to 4(f). The SEM images Figure 4 (a) seems crumpled due to the microbial attack on the tested sample and visible appearance of crack growth spread on the surface of Bio-formulated polymer nanosheet as an indicator for biodegradation process. The SEM images Figure 4 (b) gets ravaged on the surface and especially notices the rugged variation of degradation after 3 weeks. This degradation phenomenon with extension of burial period results in the decrease of molecular interaction and creates the brittleness in the prepared material.

The Figure 4 (c) a clear observation of the microbial flora presence which permeates into the Bio-

formulated polymer nanosheets and accelerates the rate of degradation process by uptaking all the nutrient source. The spindle like fiber formation can be seen clearly in Figure 4 (d, f) which weaken the interfacial adhesion and attributes to the decrease in molecular interaction of the degraded tested material. The blended plant extracts in the prepared Bio-formulated polymer nanosheets shows some difference with nonporous split like structure is indicated in the Figure 4 (e) Based on the imaging results, it is confirmed that the microbial action and chemical composition of the prepared biomaterial behavior impacts the performance of the biodegradable mechanism in soil

3.4.2. Identification of the Microbial flora Presence for degradability assessment in the soil environment

The soil microbiologically active is determined by using standard method for analyzing the presence of the microorganisms. From the Figure 4(g) to 4 (k), the presence of the microbial flora content is isolated from the tested soil which is exploited for the operation of the biodegradation of the prepared Bio-formulated polymer nanosheets. For identification of the microorganisms in Table 3, the following standard methods such as (i) Total bacterial count (Figure. 4) (j) –IS: 5402-2012 method is used and expressed as 6800000 CFU/g (ii) *Escherichia coli* –IS:5887 part 1- 1976 RA 2013 from this method , the number viable colonies of *E.coli* present in soil is isolated, identified with yellow color colonies Figure 4(h) and calculated as 74000 CFU/g (iii) *Bacillus cereus* –IS: 5887: part 6:2012, a horizontal method , the characteristic pink color colonies is observed with zone of precipitation as indication of little production of lecithinase in MYP-agar Figure 4 (g) and the presence is represented in the range of < 10 CFU/g (iv) *Pseudomonas aeruginosa*-ISO 13720-2010. The colonies that show a positive oxidase reaction in Cephalothin-sodium fusidate-cetrimide (CFC Agar) and considering its presence Figure 4 (i) as 37000CFU/g. (v) Yeast and Mold-IS 5403-1999RA 2013.by using this colony count technique the morphological examination is done and recognizes the mold colonies easily by its profuse growth of hyphae Figure 4 (k). The yeast count is also calculated by adding sodium propionate solution which inhibits mold growth in yeast extract- dextrose –chloramphenicol-agar medium at aerobic incubation for 5 days and examines its value as 140000CFU/g. From results of Figure 4 , it is confirmed that the soil is active with microbial flora content and express its full activity as a natural tool for degradation process. The bacterial, yeast and mold microbial population are found as dominantly abundant in soil with distinct ability of enhancing the degradation process in the prepared bio-formulated polymer nanosheets containing chitosan biopolymer naturally [3].

3.4.3. Energy Dispersive Scanning Electron Microscopy (EDAX) Analysis

The degraded Bio-formulated polymer nanosheets under soil environment is observed with Energy Dispersive Scanning Electron Microscopy shown in Figure 5. The degradation process is investigated after (3 to 5 weeks) due to the variation in the elemental composition of the Bio-formulated polymer nanosheets as an indication of higher degree of degradation accelerated by natural microbial flora abundantly present in the soil. The variation in elemental composition of the biodegraded Bio-formulated polymer nanosheets before and after is determined in Table 4. From the presented data, the material degradation is observed by decrease in the percentage of the carbon from 34.83 % to 5.04 %, followed by

complete decrease to 0.0% with the degradation time increased up to 5 weeks. A similar effect of decrease in oxygen percentage from 59.35% to 50.16%, which indicates that the degradation mechanism has occurred mainly owned due to the microbial attack by up taking all carbon and oxygen sources from Bio-formulated polymer nanosheets [21].

3.5. Environmental Impact and eco-efficacy of the biodegraded soil for the Eggplant (*Solanum melongena* L.) growth and nutritional yield benefit assessment

3.5.1. Seedling Growth phase Assessment

The Eggplant (*Solanum melongena* L.) is grown in the tested biodegraded soil. The initial stage development of seedling growth is observed for 2 weeks and the results represents in Table 5 (i). The percentage of seed germination is about 80 to 85% which improves the growth phase of initial stage. The seeds of eggplant growth protruding into sprouts and the seedling growth prolongs for 3 weeks. This time period requires sufficient nutrient enriched soil to attain a healthy seedling phase Figure 6(a)

3.5.2. Evaluation of the Mature Eggplant growth parameters

The Eggplant growth parameters are expressed in the Table 5(ii). The plant grows rapidly with height 138.94 ± 4.46 cm and increase in Eggplant weight 835.72 ± 6.8 gm. The number of leaves is counted in each plant and it is recorded as 42.2 ± 2.35 . The percentage of the leaf dry matter is calculated from the wet leaf weight and dried in oven for 5 hrs at 70°C until it reaches the constant weight. In this mature stage, the Eggplant produces more leaves with stronger branches to develop a healthier Eggplant [22].

3.5.3. Harvesting stage of Eggplant bearing Brinjal yield parameter analysis

The Eggplant gets mature and it bears a purple flower after 3 months in Figure 6 (b). The Eggplant requires more water and nutrients for entering into harvesting phase [23, 24]. The green color younger Brinjals are produced in 120-150 days and it reaches the ripening stage in Figure 6 (c). The mature violet color Brinjal with length range 10.9 ± 0.43 cm and the average weight of Brinjal is about 51.2 ± 2.30 gm and recorded in the Table 6(i). The calculation is done with the experimentation of the growth of 35 Eggplants in the plot area and observe the yield around 45 kg per plot. The final Brinjal yield per hectare is estimated around 430-450 kg /hectare is produced from the biodegraded soil.

3.5.4. Determination of the nutrient quality for the Eggplant Brinjals cultivated from the biodegraded soil

The nutritional quality is assessed for the evaluation of the Eggplant Brinjals and it is represented in the Table 6(ii). 500gms of the brinjals are taken from the harvested Eggplant grown in the tested biodegraded soil. The Total Soluble Solid (TSS) of the Eggplant is determined by using IS 13815-2010 RA 2005 standard refractometric method. The average TSS value is expressed in the range of 2.51-3.51 for the tested COBH2(2009) brinjal variety. The protein content for the Eggplant (1.16 g/100g) which is obtained by kjeldahl standard method (IS7219-1973 RA 2015). The carbohydrate value is calculated as 5.79 g/100g based on Food energy method of analysis and conversion factors (FAO). The mineral potassium

content present in the Eggplant is measured as 3101mg/kg by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) with Standard operation method (BVILCH/INS/SOP-053). From this study, the result is analyzed for nutritional fact and the obtained value is similar to that of standard value and its health benefits [25].

4. Conclusion

The results from SEM and EDAX analysis indicated that the developed Bio-formulated polymer nanosheets were degraded completely after 5 weeks examination. Based on results, the introduction of CNSL reduced the overall migration value to 3.77mgdm^{-2} can be significantly used as packaging material for food products. The variation in the elemental composition of the Carbon wt% decreased completely from 34.83 % to 0% and also oxygen wt% slightly varied from 59.35 to 50.16% were observed in the degraded Bio-formulated polymer nanosheet for 5 weeks and surface morphology analysis which reflects an excellent eco-friendly packaging material. Moreover, the tested biodegraded soil was evaluated for the seedling growth stage and nutritional analysis of the Eggplant (*Solanum melongena* L.). The experimental result data of this research, demonstrates that developed Bio- polymer nanosheets packaging material can be predicted as a successful eco-characteristic material by overcoming the toxicity of the solid waste accumulation for environmental safety.

Declarations

Funding

The funding from Anna university, Chennai. Under the scheme Anna Centenary Research Fellowship (ACRF) to carry out the research.

Acknowledgement

The authors are feeling grateful to the Crystal Growth Centre, Anna university, Chennai for providing facilities to support this research study. One of the authors Ruby Nirmala Mary Thomas would like to thank Anna University for the award of Anna Centenary Research Fellowship (ACRF) to carry out the research.

Conflict of Interest

The authors declare no conflicts of interest.

Ethics approval

Not Applicable

Consent to participate

I understand that all information I provide for this study will be treated confidentially.

Consent to Publish

I give my consent for the publication of identifiable details, which can include photograph(s) and data details within the research article to be published in the Waste and Biomass Valorization. Therefore, anyone can read material published in the Journal.

Availability of data and material

Data available in a public (institutional, general or subject specific) repository that issues datasets with DOIs (non-mandated deposition).

Code availability

Not Applicable

Authors' contributions

T. Ruby Nirmala Mary

(First author) : Data curation, Formal analysis, Investigation, Methodology, Resources, formulating figures and table, formatting the article writing – original draft

Dr. Jayavel Ramasamy

(Corresponding author) : Conceptualization, Project administration, Supervision, Validation, Visualization, Review the article & Editing

References

1. Muncke, J., Andersson, A.-M., Backhaus, T., Boucher, J.M., Chevrier, B., Carney A., Castillo C., J., Demeneix, B.A., Jorge, A., Emmanuel, J.-B., Fini, D., Gee, B., Geueke, K., Groh, J.J., Heindel, J., Houlihan, C.D., Kassotis, Carol, F., Kwiatkowski, Lisa, Y., Lefferts, M.V., Maffini, O.V., Martin, J.P., Myers, A., Nadal, C., Nerin, K.E., Pelch, Seth, Rojello Fernández, Robert, Sargis, M., Soto, A.M., Trasande, L., Vandenberg, L.N., Wagner, M., Wu, C.: R. Thomas Zoeller, Martin Scheringer.: Impacts of food contact chemicals on human health: a consensus statement. Environ. Health. **19**, 25 (2020). <https://doi.org/10.1186/s12940-020-0572-5>
2. Kamdem, D.P., Shen, Z., Nabinejad, O.: Zuju Shu .: Development of biodegradable composite chitosan-based films incorporated with xylan and carvacrol for food packaging application. Food Packag. Shelf Life. 21 (2019). <https://doi.org/10.1016/j.fpsl.2019.100344>

3. Cornelia Vasile, D., Pamfil, M., Rapa: Raluca Nicoleta Darie-Nița, Amalia Carmen Mitelut, Elena Elisabeta Popa, Paul Alexandru Popescu, Mihaela Cristina Draghici, Mona Elena Popa.: Study of the soil burial degradation of some PLA/CS biocomposites. *Compos. B Eng.* **142**, 251–262 (2018). <https://doi.org/10.1016/j.compositesb.2018.01.026>
4. Ediyilyam, S., George, B., Shankar, S.S., Dennis, T.T., Waclawek, S., Černík, M., Padil, V.V.T.: Chitosan/Gelatin/Silver Nanoparticles Composites Films for Biodegradable Food Packaging Applications. *Polymers.* **13**, 1680 (2021). <https://doi.org/10.3390/polym13111680>
5. Hossein Haghighi, R.D., Leo, E., Bedin, F.P., Siesler, H.W.: Andrea Pulvirenti Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. *Food Packag. Shelf Life.* **19**, 31–39 (2019). <https://doi.org/10.1016/j.fpsl.2018.11.015>
6. Martău, G.A., Mihai, M., Vodnar, D.C.: The Use of Chitosan, Alginate, and Pectin in the Biomedical and Food Sector—Biocompatibility, Bioadhesiveness, and Biodegradability. *Polymers.* **11**, 1837 (2019). <https://doi.org/10.3390/polym11111837>
7. Krystyjan, M., Khachatryan, G., Grabacka, M., Krzan, M., Witczak, M., Grzyb, J., Woszczak, L.: Physicochemical, Bacteriostatic, and Biological Properties of Starch/Chitosan Polymer Composites Modified by Graphene Oxide, Designed as New Bionanomaterials. *Polymers.* **13**, 2327 (2021). <https://doi.org/10.3390/polym13142327>
8. Victor, G.L., Souza, Joao, R.A., Pires, C., Rodrigues, I.M., Coelho: Ana Luísa Fernando.: Chitosan Composites in Packaging Industry—Current Trends and Future Challenges. *Polymers.* **12**, 417 (2020). <https://dx.doi.org/10.3390/polym12020417>
9. Manokaran Vadivel, M., Sankarganesh, J.D., Raja, J., Rajesh, D., Mohanasundaram, M., Alagar: Bioactive constituents and bio-waste derived chitosan / xylan based biodegradable hybrid nanocomposite for sensitive detection of fish freshness. *Food Packag. Shelf Life.* **22**, 100384 (2019). <https://doi.org/10.1016/j.fpsl.2019.100384>
10. Wenqian Wang, Q., Meng, Q., Li, J., Liu, M., Zhou, Z., Jin, K., Zhao: Chitosan Derivatives and Their Application in Biomedicine. *Int. J. Mol. Sci.* **21**(2), 487 (2020). <https://doi.org/10.3390/ijms21020487>
11. Pei Gie Gan, Sam, S.T., Abdullah, M.F., Omar, M.F., Wai Kian Tan : Water resistance and biodegradation properties of conventionally-heated and microwave-cured cross-linked cellulose nanocrystal/chitosan composite films. *Polym. Degrad. Stab.* 188:109563. (2021). <https://doi.org/10.1016/j.polymdegradstab.2021.109563>
12. Elena Stoleru, E.G., Hitruc, C., Vasile, L., Oprica: Biodegradation of poly(lactic acid)/chitosan stratified composites in presence of the *Phanerochaete chrysosporium* fungus. *Polym. Degrad. Stab.* **143**, 118–129 (2017). <https://doi.org/10.1016/j.polymdegradstab.2017.06.023>
13. Valentina Siracusa: Microbial Degradation of Synthetic Biopolymers Waste. *Polymers.* **11**(6), 1066 (2019). <https://doi.org/10.3390/polym11061066>
14. Ayilara, M.S., Olanrewaju, O.S., Olubukola Oluranti Babalola, Olu Odeyemi. Waste Management through Composting: Challenges and Potentials. *Sustainability.* **12**: 4456. (2020).

<http://dx.doi.org/10.3390/su12114456>

15. Katarzyna Chojnacka, K., Moustakas: AnnaWitek-Krowiak: Bio-based fertilizers: A practical approach towards circular economy. *Bioresour Technol.* **295**, 122223 (2020).
<https://doi.org/10.1016/j.biortech.2019.122223>
16. Massimo Malerba, R., Cerana: Recent Advances of Chitosan Applications in Plants. *Polymers.* **10**, 118 (2018). <https://dx.doi.org/10.3390%2Fpolym10020118>
17. Serrano-Ruiz, H., Martin-Closas, L., Pelacho, A.M.: Application of an in vitro plant ecotoxicity test to unused biodegradable mulches. *Polym. Degrad. Stab.* **158**, 102–110 (2018).
<https://doi.org/10.1016/j.polymdegradstab.2018.10.016>
18. Salma Sultana, M., Islam, M.A., Khatun, M.A., Hassain: Roksana Huque: Effect of Foliar Application of Oligo-chitosan on Growth, Yield and Quality of Tomato and Eggplant. *Asian J. Agric. Res.* **11**(2), 36–42 (2017). <http://dx.doi.org/10.3923/ajar.2017.36.42>
19. Maria Rapa, A.C., Miteluț, E.E., Tanase, E., Grosu, P., Popescu, M.E., Popa, J.T., Rosnes, M., Sivertsvik: Raluca Nicoleta Darie-Nița, Cornelia Vasile : Influence of chitosan on mechanical, thermal, barrier and antimicrobial properties of PLA-biocomposites for food packaging. *Compos. B Eng.* **102**, 112–121 (2016). <https://doi.org/10.1016/j.compositesb.2016.07.016>
20. Lv, S., Zhang, Y., Gu, J., Tan, H.: Biodegradation behavior and modelling of soil burial effect on degradation rate of PLA blended with starch and wood flour. *Colloids Surf. B Biointerfaces.* **159**, 800–808 (2017). <https://doi.org/10.1016/j.colsurfb.2017.08.056>
21. Mashael Alshabanat: Morphological, thermal, and biodegradation properties of LLDPE/treated date palm waste composite buried in a soil environment. *J. Saudi Chem. Soc.* **23**, 335–364 (2019).
<https://doi.org/10.1016/j.jscs.2018.08.008>
22. Kutaiba YuserAied, Z., Wahab, RezuwanHj: Kamaruddin, Abd Razak Shaari.: Growth response of eggplant (*Solanum melongena* L.) to shading and cultivation inside greenhouse in a tropical region. *Int. J. Sci. Eng. Res.* **8**(5), 89–101 (2017)
23. Dash, S.P., Singh, J., Sharma, D.: Morphological characterization of brinjal (*Solanum melongena* L.) germplasm. *J. pharmacogn and Phytochem* **8**(2), 1574–1578 (2019)
24. Maysoun, M., Saleh, O., Muhra: Zaeda Alsayd Suliman: Selection criteria for yield in eggplant (*Solanum melongena* L.). *Hortic. Biotechnol. Res.* **5**, 01–03 (2019). doi:10.25081/hbr.2019.v5.5454
25. Naeem, M.Y., Ugur, S.: Nutritional Content and Health Benefits of Eggplant. *Turkish J. Agric. - Food Sci. Technol.* **7**(sp3), 31–36 (2019). <https://doi.org/10.24925/turjaf.v7isp3.31-36.3146>

Tables

Table 1. The overall migration values for the prepared Bio- formulated polymer Nano sheets (mgdm⁻²).

Samples	Overall migration value(mg/dm ²)
1-CTS	1.4±1
2-CTS-CNSL	3.77±1
3.CTS-CNSL-Ironoxide Np	25.4±1
4. CTS-CNSL-Ironoxide Np-PE 1	26.85±1
5. CTS-CNSL-Ironoxide Np-PE 2	46.4±1
6. CTS-CNSL-Ironoxide Np-PE 3	50.9±1

Note : Values are expressed as mean±Standard deviation (n=2)

Table 2. The determination of the weight mass loss % variation for the prepared Bio-formulated polymer nanosheets containing the chitosan biopolymer matrix (CTS), Cashew nut shell liquid (CNSL), Iron oxide nanoparticles and the plant extracts of (4) *Mentha spicata* L. (PE1) (5) *Vitex negundo* L. (PE2) (6) and with *Eucalyptus globulus* M. (PE3) after 5 weeks of biodegradation process activated by soil microbial communities

Samples	Control (0 days)	1 st week (7 days)	2 nd week (14 days)	3 rd week (21 days)	4 th week (28 days)	5 th week (35 days)
CTS	0	20	33.33	66.66	80	93.66
CTS-CNSL	0	13.33	26.66	46.66	60	80.00
CTS-CNSL- Iron oxide NP	0	0	20	60	73.33	86.66
CTS- CNSL- Iron oxide NP -PE1	0	0	26.66	53.33	66.66	86.66
CTS-CNSL- Iron oxide NP -PE2	0	0	20	53.33	80	93.33
CTS-CNSL- Iron oxide NP -PE3	0	6.66	26.66	40	66.66	80

Table 3. The microbiological examination of the total microbial population presence in the biodegraded soil quantity 1kg used for the biodegradation of the prepared Bio-formulated polymer nanosheets.

S. No	Test Parameters	Result
	Microbial flora presence	CFU/g
1	<i>Bacillus cereus</i>	<10
2	<i>Escherichia coli</i>	74000
3	<i>Pseudomonas aeruginosa</i>	37000
4	Total Bacterial count	6800000
5	Yeast and Mold	140000

Table 4. The determination of the elemental composition Wt % content for the biodegraded Bio-formulated polymer nanosheets before and after 5weeks in soil environment

Elemental composition of the prepared Bio- formulated Polymer Nano Sheets	Before degradation Wt%	After degradation Wt% (3 weeks)	After degradation Wt% (4 weeks)	After degradation Wt% (5 weeks)
Wt%				
C	34.83	5.04	0.00	0.00
O	59.35	59.83	52.15	50.16
Si	3.91	17.15	21.19	22.18
Ca	1.36	5.31	7.63	8.11
Mg	0.51	—	0.73	0.65
Fe	0.04	—	6.45	6.82

Table 5. Initial development of the Eggplant from seedling stage into healthier plant growth in the biodegraded tested soil containing the nutrient source from Bio-formulated polymer nanosheets

(i) Initial development of the Eggplant seedling growth		
S. No	Seedling Growth parameters	Results
1	Seed Germination %	80±5.77
2.	Mean daily Germination upto14 days	0.57±0.004
3.	Moisture content % (Initial Sprouting Phase)	36.76±1.79
(ii) Determination of the Eggplant growth Parameters		
4.	Egg plant height (cm)	138.94±4.46
5.	Number of leaves per plant	42.2±2.35
6.	Initial stage Stem diameter (mm)	12.42±0.24
7.	Final stage Stem diameter (mm)	15.38±0.37
8.	Number of nodes	37.2±1.71
9.	Eggplant weight(gm)	836.72±6.8
10.	Percentage of leaf dry matter (%)	17.20±0.74

Note : Values are expressed as mean±Standard deviation (n=5)

Table 6. The observation analysis for the Eggplant Brinjal Yield and Nutritional quality of the brinjal at harvesting stage from the biodegraded soil

(i) Evaluation of the Brinjal yield parameters			
S. No	Yield parameters	Results	
1.	Brinjal weight(gm)	51.2±2.30	
2	Brinjal length (cm)	10.9±0.43	
3	Brinjal Girth(cm)	9.53±0.17	
4	Number of Brinjals per plant	25.66±2.4	
5	Brinjal yield per plant (kg)	1.29±0.06	
6	Brinjal yield per plot (kg)	45.38±2.41	
7	Brinjal yield per hectare(kg/ha)	430-450 (approx.)	
Note: Values are expressed as mean±Standard deviation (n=5)			
(ii) Nutritional quality of the Brinjal at harvesting stage			
S.No	Test Parameters	Unit of Measurement	Results
1	Total Soluble Solid @ 20° C	%	2.51-3.5 (approx.)
2	Protein (N×6.25)	g/100g	1.16
3	Carbohydrate	g/100g	5.79
4	Potassium	mg/kg	3101

Figures

Figure 1

Visual transparency (a) chitosan biopolymer matrix and (b) Bio-formulated polymer nanosheets developed with the blends of chitosan/CNSL /iron oxide nanoparticles and plant extracts. The SEM images of the surface morphology for the bio-formulated polymer nanosheets (c) control-chitosan biopolymer matrix (d) the prepared Bio-formulated polymer nanosheets containing the CNSL/ iron oxide nanoparticles with plant extracts in Chitosan biopolymer matrix exhibits a uniform dispersion as glossy appearance material

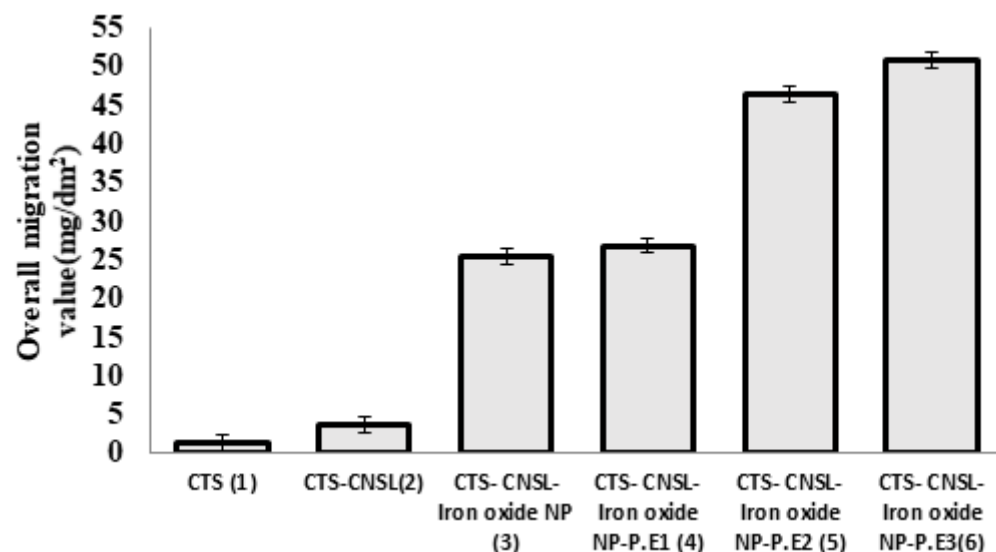


Figure 2

The overall migration test for the prepared Bio- formulated polymer nanosheets represents the lesser migration of the components after blending CNSL (2), a natural binder used in the formulation composition containing the iron oxide nanoparticles (3) plant extract of (4) *Mentha spicata* L. (P.E1) (5) *Vitex negundo* L. (P.E2) (6) and with *Eucalyptus globulus* M. (P.E3). The Error bars with indication of Standard deviation (SD) and difference in the opacity value significantly mean ($p < 0.05$).

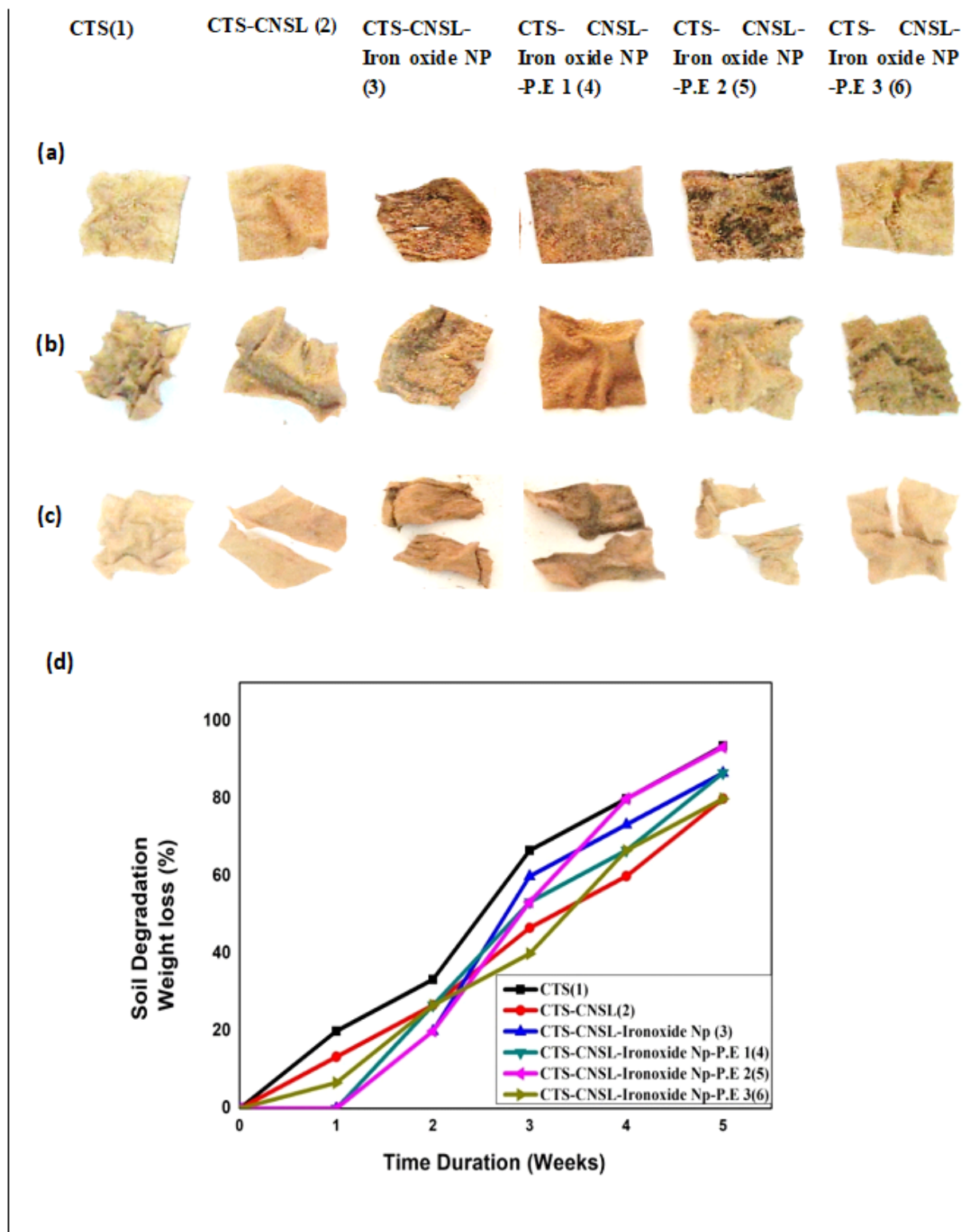


Figure 3

The visible observation of the biodegraded Bio-formulated polymer nanosheets after 5 weeks degradation period (a) Initial weight loss degradation starts (b) The soil microbes uptake carbon material source from Bio-formulated polymer nanosheets corresponding to the breakdown of the prepared material (c) The final step results in cleavage process of complete degradation (d) The weight loss percentage as a degree of degradation of the Bio-formulated polymer nanosheets evaluation for biodegradation process up to 5

weeks. The representation clearly explains more intense in the degradation pattern after 3 weeks. The higher percentage of mass loss registers for all the prepared samples about 80 to 93% for burial period of 5 weeks

Figure 4

The surface morphological examination of the Bio-formulated polymer nanosheets (a) Visible appearance of the crack growth formed due to microbial growth on the surface of prepared sample as indicator of degradation process (b) The results shows the rugged variation in surface after 3weeks which happens with the decrease of molecular interaction and creates the brittleness in the prepared material (c) Soil Microbial presence observed on its surface for accelerating natural way of degradation mechanism (d) Spindle-like formation enhances degradation process by weakening the interfacial adhesion (e) Identification of split like structure (f) Degraded material impact of mostly affected by microbial soil flora on the surface of the Bio-formulated polymer nano sheets. The observation of the microbial flora presence promotes the natural soil degradation mechanism for decomposition of the Bio-formulated polymer nanosheets (g) *Bacillus cereus* (h) *Escherichia coli* (i) *Pseudomonas aeruginosa* (j) Total Bacterial count (k) Yeast and Mold. The microbial population identified as dominantly abundant in soil with distinct behavior of accelerating the degradation process naturally.

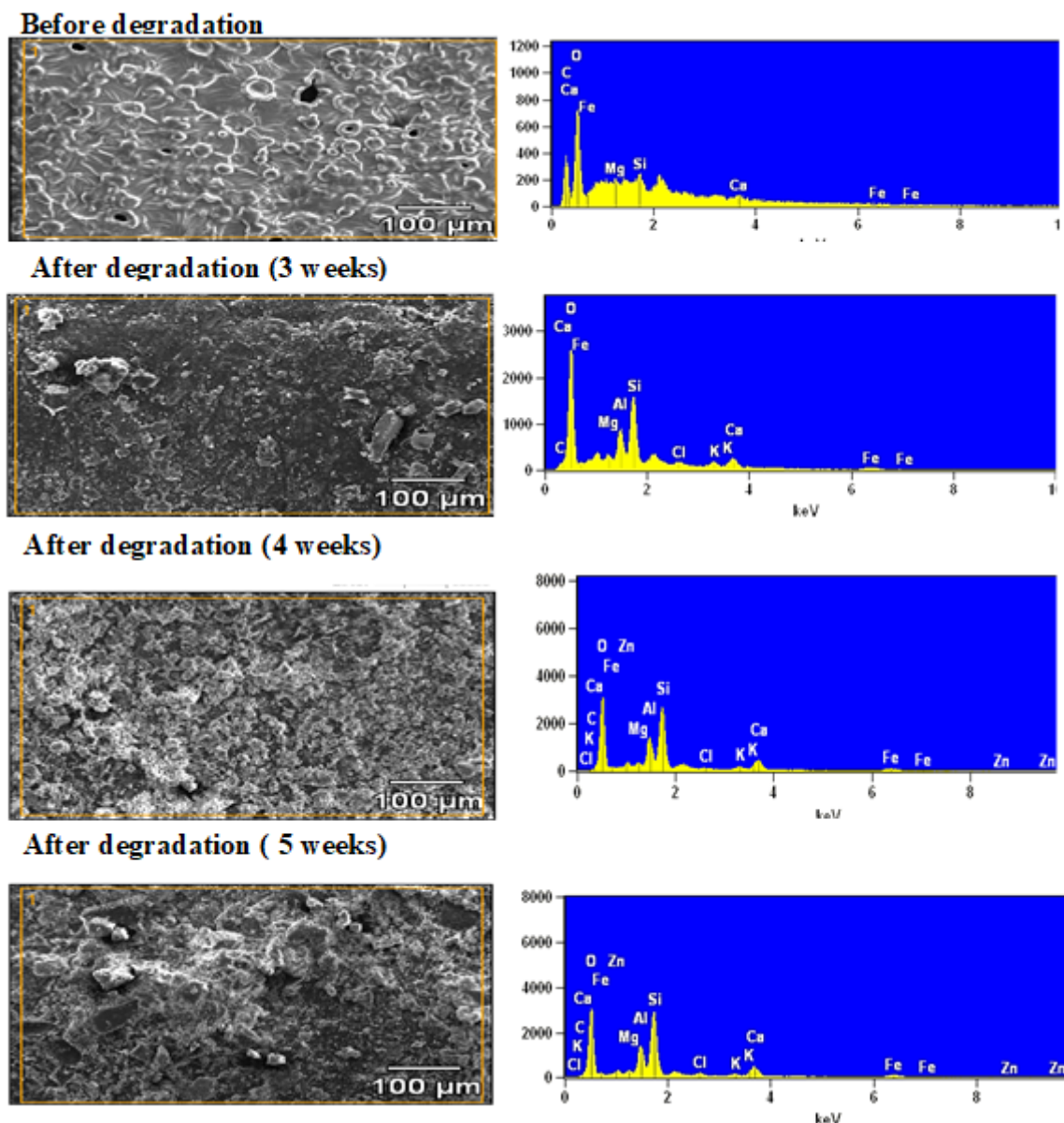


Figure 5

Elemental composition Analysis of the degraded Bio-formulated polymer nanosheets for a period of 5weeks. The degradation at initial weeks, the carbon source up taken by soil microbes getting decreased in the percentage of the carbon from 34.83 % to 5.04 % (3 weeks), followed by complete decrease to 0.0% (5 weeks). The effect of oxygen percentage reduction from 59.35% to 50.16%, which indicates the prepared material degradation.

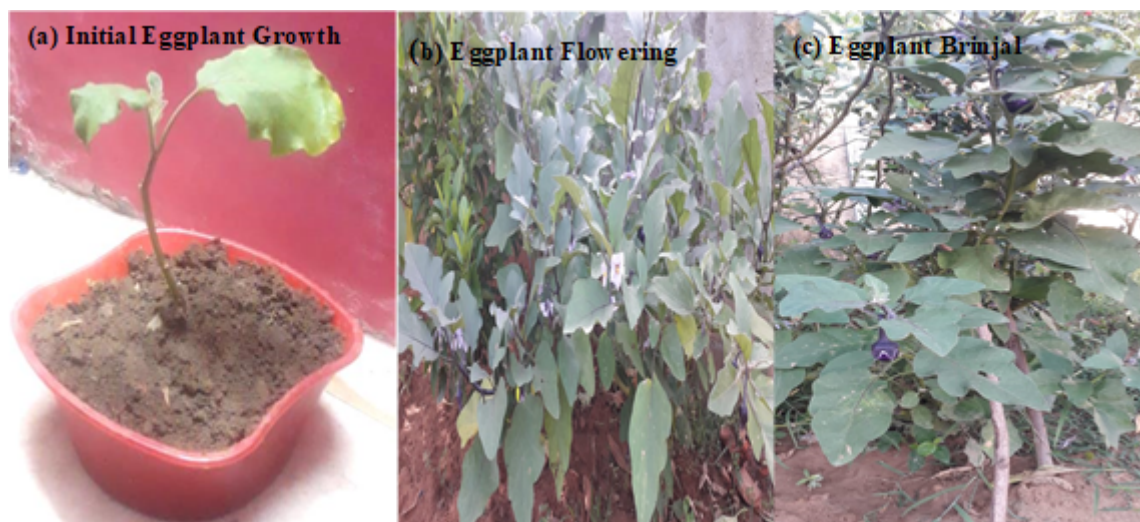


Figure 6

The growth stages of the Eggplant (*Solanum melongena* L.) for assessment of growth parameters (a) Seedling growth- The percentage of seed germination is estimated as 80 to 85% results in promoting the initial growth stage. The seeds protrude into sprouts and the healthy seedling growth prolongs for 3 weeks (b) Small purple color flowers produced in the Eggplant (c) Violet color Brinjal bearing stage of Eggplant