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Organic fertilizer integrated with marine waste derived $CaCO_3$ nanocarrier system: A focus on enhanced yield and quality in tomato cultivation

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Tomatoes are rich in lycopene, β-carotene, ascorbic acid and other mineral sources including phosphorus, potassium, zinc, magnesium and iron. Major constraints in tomato cultivation were high cost, poor cultivation due to adverse weather conditions, pest attacks, microbial infections and nutritional deficiency complications. Conventional fertilizers, pesticides, fungicides and growth regulators are effective at higher concentration, which induces specific toxic effects on soil fertility, plant yield and also affects the health status of humans, animals and soil associated microbes. The use of organic fertilizers to meet the soil nutrient demand increases the acidity of soil affecting plant growth, which turned the focus of researchers towards nanofertilizer. The present study focuses on synthesis of marine waste derived $CaCO_3$ nanoparticles formulated with azadirachtin and panchakavya emulsion to develop a $CaCO_3$ nanofertilizer. $CaCO_3$ nanofertilizer developed through this study was investigated for its material properties and behavioral traits. Further, the in-vitro antifungal impact of the $CaCO_3$ nanofertilizer was examined, and it was sprayed on tomato plants via foliar spray. $CaCO_3$ nanofertilizer effectively inhibited fusarium wilt causing plant fungal pathogen and also exhibited enhanced growth and yield of tomatoes against pest attack and nutritional deficiency with effect to foliar treatment. Also, $CaCO_3$ nanofertilizer enhanced the total carotenoid level and essential nutritional minerals in fruit yield of tomatoes. Overall, fabricated $CaCO_3$ nanofertilizer exhibits synergistic role of fertilizer, pesticide, fungicide and growth regulator in tomato cultivation. It suggests that, $CaCO_3$ nanofertilizer generated from renewable biowaste will become the innovative platform for sustainable agriculture.

Keywords $CaCO_3$ nanoparticles, Azadirachtin emulsion, Panchakavya extract, Nanofertilizer, Foliar treatment and tomato crops

Consumption of agricultural products like fruits, vegetables, nuts, whole grains and legumes are essential for a healthy life style, which prevents the incidence of non-communicable human disorders such as malignancy, cardiac diseases, diabetics, stroke and also protects from communicable infectious diseases[1]. Outburst of global population increased the demand for food provoking use of chemical fertilizers to enhance the crop productivity which affects soil fertility. Population increase has led to child malnutrition due to insufficient intake of nutrient rich vegetables in both developed and developing countries due to poor productivity, cost effect and other drawbacks in global vegetable cultivation[2]. Global vegetable insufficiency can be overcome by the growth of nutrient enriched regional vegetables, which will nurture the vegetable demand of local population at low cost as there is no need for long-term transport[3]. However, cultivation of regional vegetables also suffers certain obstacles such as pest attacks, microbial infections, nutritional deficiency and adverse weather conditions such as heavy rain, increased temperature etc.[4–7].

Use of conventional fertilizers, pesticides and fungicides in large quantities in the agricultural field disturbed soil mineral balance by accumulation of heavy metals (like cadmium, lead and arsenic) and hazardous compounds, affecting the soil environment and water resources. Toxic byproducts from insecticides and fungicides

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present in the cultivated vegetables causes toxic disorders to the consumed human beings[8,9]. The above draw backs has increased the need for ecofriendly nontoxic fertilizer with high nutrient use efficiency. Recently, use of organic fertilizers mostly composed or derived from waste and byproducts of plants, animals and microorganisms as an ecofriendly approach. The organic fertilizers consist of bioactive nutrients and minerals that were principally released to the soil and plant environment for the growth and development of plants to achieve a better yield. The utilization of organic compounds enriched with nitrogen (N), phosphorous (P) and potassium (K) by the plants and soil found microbes significantly enhances plant growth and yield through biomass production, photosynthesis and nitrogen fixation[10,11].

Panchakavya is an excellent non-toxic and less expensive organic fertilizer, which boosts the growth and yield of agricultural crops. Panchakavya was prepared from the cow's byproducts such as dung, urine, milk, curd and ghee along with the formulation of organic compounds such as tender coconut water, jaggery and well ripened banana in the water suspension. The organic compounds in the formulation of panchakavya are significantly enriched with primary nutrients (NPK), sodium, calcium, fatty acids and alkanes. Inclusive of the above minerals, panchakavya consists of plant growth regulating hormones such as Indole-3-acetic acid (IAA) and Gibberellic acid (GA) (Tamil Nadu Agricultural University, Coimbatore, Tamilnadu, India). The nutritional composition of the panchakavya significantly raises the production and shelf life of plant yield through pest, pathogen and insecticide tolerance in addition to the better nutritional uptake[12]. On the other hand, azadirachtin is an active organic compound derived from the source *Azadirachta indica,* which plays the role of pesticides, insecticides and fungicides, efficiently to protect plants from possible biotic stress factors[13,14]. Use of organic fertilizers suffers certain limitations such as degradation of the nutrients and growth promotors by biotic and abiotic stress factors including temperature, increased salinity, acidic pH, oxidative stress and biodegradation of compounds. Also, the extreme usage of organic fertilizers like panchakavya induces toxic effect in the soil and plant environment such as extreme acidic, significantly affects the plant growth and yield[15]. Also, the minimal usage of organic fertilizers causes insufficient nutritional uptake, growth regulation and pest/microbial tolerance. The above drawbacks were overcome with use of nanofertilizers, which exhibited smart and sustained agricultural practices for improved progress in plant cultivation and yield. Nanomaterials were mostly used as a carrier system for the slow and controlled release of the fertilizer to the plants in a prolonged manner, which is significantly helpful for the enhanced yield. Additionally, the degradation of the nanostructures, also released minerals and nutrient elements that were helpful for agricultural production. The present work plan desired to fabricate $CaCO_3$ nanofertilizer by the formulation of panchakavya extract and azadirachtin emulsion with the marine waste derived $CaCO_3$ nanoparticles. Solubility of nanofertilizer was optimized by the composition of hydrophilic panchakavya and hydrophobic azadirachtin emulsion in the $CaCO_3$ nanocarrier system. The fabricated nanofertilizer has been applied for the cultivation of Indian traditional cherry tomatoes by foliar approach. Every year India largely cultivates and consumes tomatoes for traditional cooking, industrial manufacturing and global exportation. But, the cultivation of tomatoes was majorly affected by nutritional deficiency[16], pest attacks[17] majorly with fusarium wilt[18]. Hence, this work plan desired to evaluate the potential of $CaCO_3$ nanofertilizer on tomato cultivation and assess the antifungal potential against wilt disorder causing fungal pathogens. Calcium (Ca) is an essential nutrient for the plant growth and development facilitating cell wall construction and resist environmental stress. Calcium also plays vital role in regulating photosynthetic pathway. Nano calcium carbonate particles are quickly absorbed by plants due to its smaller size and strong adhesion property. The $CaCO_3$ nanoparticles consist of calcium, carbon, and oxygen in their elemental composition. Foliar spray of $CaCO_3$ nanoparticles facilitates quick absorption of Ca ions in the leaf surface and root. In addition, the porous nature of $CaCO_3$ nanoparticles facilitates loading of organic fertilizer Panchakavya extract and pesticide Azadirachtin emulsion as a carrier system allowing slow and sustained release of fertilizer to plants without any toxic effect. Here, the synergistic effect of the $CaCO_3$ nanoparticles, panchakavya extract, and azadirachtin emulsion present in the nanofertilizer play multiple potentials as fertilizer, insecticide, and fungicide for the sustainable and ecofriendly way for tomato cultivation as a solution for the drawbacks in the conventional process.

Experimental section

Required materials and sample collection

All the chemicals, reagents and biological supplements such as Azadirachtin ($C_{35}H_{44}O_{16}$), Czapek-dox broth and potato dextrose agar were purchased from Sigma Aldrich and used without any further purification. Conch shell waste was derived from the coastal area of Thondi, Ramanathapuram, Tamilnadu, India (9.74° N, 79.01° E). Panchakavya was purchased from the Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India. The vermicompost and cocopeat were obtained from the SethuBhaskara Agricultural College and Research Foundation, Tamilnadu, India.

Synthesis of $CaCO_3$ nanoparticles

Synthesis of $CaCO_3$ nanoparticles was done in four phases based on our previous work[19]. In phase 1, the surface sterilized and well dried conch shell waste was grained into the form of powder and further, it was subjected to plenary ball milling for 60 min at 250 rpm. The 3:1 (w/w) ratio of citric acid and above conch shell powder was treated for overnight in the aqueous medium at 37 °C and the yield conch shell powder was then dried and calcinated at 80 °C and 750 °C respectively in phase 2. Phase 3 involves the removal of impurities from the conch shell powder by treating the above separately with NaOH (1 M) and HCl (10%) thrice, followed by drying and calcination at the same temperature as phase 2. The obtained conch $CaCO_3$ was subjected to in-situ deposition in phase 4, to obtain $CaCO_3$ nanoparticles. Briefly, the mixture consists of 1:100 (w/w) ratio of chitosan in acetic acid (5%) and the $CaCO_3$ in Concentrated HCl, which were blended with sodium carbonate at 50 °C for 0.25 h. The blended mixture of the above was rested for overnight at 37 °C to attain the in-situ deposition. Finally, the

deposited yield was further dried at 80 °C followed by calcination at 750 °C and the yield of $CaCO_3$ nanoparticles was stored in a vacuum container.

Cold extraction of panchakavya

Based on the fermentation of cow's byproducts and other organic formulations in the panchakavya, it was observed that panchakavya is greatly enriched with certain essential nutrients, like growth regulators/promotors and growth promoting microbial loads for the plants. However, while considering the nanoformulation, the solid suspensions present in the panchakavya may result in micro sized particles of nanofertilizer. In order to avoid the above drawback, the cold digestion was carried out to remove the solid particles and to avail all the nutritious components of panchakavya in the aqueous form. Here, the extraction setup was maintained in the ice bath to protect the heat sensitive vital nutrients, growth promotors and other bioactive compounds present in the panchakavya fertilizer. For the cold extraction, the mixture of 1:1 ratio of panchakavya and distilled water (v/v) was kept under constant stirring overnight under dark condition and further homogenized at 20 kHz for 60 min. After that, the extract was filtered twice with crude filter paper and followed by vacuum filter to separate the solid and liquid phases. Finally, the filtered extract panchakavya was stored at room temperature in dark condition.

Fabrication of $CaCO_3$ nanofertilizer

The $CaCO_3$ nanofertilizer was fabricated by the formulation of panchakavya and neem emulsion with the $CaCO_3$ nanoparticles. In detail, 1 g of $CaCO_3$ nanoparticles in 50 mL of distilled water was kept under constant stirring for 1 h to get the uniformed dispersion. After that 10 mL panchakavya extract and 1 mL of azadirachtin emulsion (dissolved using 100 µL of citronellyl acetate) were added drop wise together with the dispersion of $CaCO_3$ nanoparticles. The above mixture was stirred for 30 min and it was then transferred to the rotatory orbital shaker at 37 °C for overnight at 150 rpm. After the agitation process, the yield sample was collected by centrifugation and lyophilized at − 80 °C. The prepared $CaCO_3$ nanofertilizer was then stored in an air tight vacuum container until further use.

Fertilizer loading efficiency

The fertilizer loading efficiency was calculated by the concentration of the fertilizer (panchakavya and azadirachtin emulsion) loaded in the $CaCO_3$ nanoparticles. The percentage of loaded fertilizer was calculated by the unloaded remaining fertilizer concentration present in the supernatant collected after the fabrication $CaCO_3$ nanofertilizer. The absorbance value of the fertilizer in the supernatant was matched with the slope absorbance value of the standard calibration graph plotted for the two-fold concentrations of the fertilizer mixture (panchakavya and azadirachtin emulsion) from 25 to 200 µL using UV–visible spectrometer at 300 nm. The slope value determines the concentration of the fertilizer present in the supernatant and the percentage of fertilizer loading efficiency was calculated using the following formula model

$$\text{Loding efficieny}\,(\%) = \frac{\mathrm{F}_{total} - \mathrm{F}_{free}}{\mathrm{F}_{total}} \times 100 \tag{1}$$

whereas the F_{total}—is the total concentration of the fertilizer taken for the fabrication of the nanofertilizer and F_{free}—is the unloaded concentration of the fertilizer present in the supernatan[1,20].

Swelling rate of the nanofertilizer

The swelling ratio was evaluated by tipping the $CaCO_3$ nanofertilizer in the separate falcon tubes containing the distilled water and ground water respectively. For that, 30 mg of the nanofertilizer was treated with 30 mL of distilled water and ground water individually. The samples were then kept for swelling up to 24 h, further the swelling ratio and equilibrium water content was calculated using the following formula models at every 4 h

$$\text{Swelling ratio} = \frac{F_w - F_d}{F_d} \tag{2}$$

$$\text{Equilibrium water content} = \frac{F_w - F_d}{F_w} \times 100 \tag{3}$$

whereas the F_w—is the wet and F_d—is the dry weight of the nanofertilizer[19,21].

Water adsorption capacity

Water adsorption capacity is about the capacity of the sample to adsorb the moisture content for certain period. To calculate the water adsorption capacity of the nanofertilizer, three reweighed beakers was individually added with 30 mg of the sample and kept in a desiccator under the moisture environment for seven days. Followed by the beakers with the sample were reweighed and the water adsorption capacity of the nanofertilizer was calculated using the following formula

$$\text{Water adsorption capacity}\,(\%) = \frac{W_{moist} - W_{dry}}{W_{dry}} \times 100 \tag{4}$$

where the W_{dry}—is the mass of the sample before water adsorption and W_{moist}—is the mass of the sample after water adsorption[22].

Fertilizer release rate

The fertilizer release profile was carried out in two various aqueous system, the ground water and ground water adulterated with the pot mixture used in the grow bags for the tomato cultivation. To calculate the fertilizer release from the $CaCO_3$ nanoparticles, 30 mg of the nanofertilizer was added with 200 mL of ground water and ground water with pot mixture separately was stirred under optimum rpm at room temperature. The above setup was maintained for a week and the percentage of release was calculated by collecting the supernatant from the above setup after 1, 3, 5 and 7 days of interval respectively. The percentage of fertilizer release rate was calculated using the following formula model

$$\text{Fertilizer release rate (\%)} = \frac{N_{released}}{N_{loaded}} \times 100 \tag{5}$$

where $N_{released}$—is the concentration of the fertilizer present in the supernatant collected from the fertilizer release model systems and N_{loaded}—is the concentration of the fertilizer loaded with the nanocarrier system[23].

Characterization

The material characterization studies were performed to demonstrate the crystalline structure, morphology, size, functional and physiochemical aspects of the samples including nanomaterials and the fertilizer samples. X-ray diffraction studies were carried out to find the crystalline phase of the $CaCO_3$ nanoparticles alone and $CaCO_3$ nanofertilizer using Powder X-ray Diffractometer (HTK 1200N—Bruker D8 Advance). Fourier transform infrared (FTIR) spectroscopy results the functional frequency of the nanomaterials and the fertilizer compounds from 400 to 4000 cm^{-1} (Thermo Nicolet 380). UV–visible spectra of the nanomaterials and fertilizer compounds results the specific absorbance of the samples, which is also been used to evaluate the loading and sustained release of the fertilizer compounds (3092, Lab India). The average particle size and the zeta charge of the nanostructures were analyzed using dynamic light scattering (DLS) based on the Brownian motion (NanoPlus, Micrometrics) and zeta analyzer (Zetasizer, ver 8.02) respectively. Morphology of the nanostructures was examined through field-emission scanning electron microscope (FE-SEM) along with energy dispersive X-ray (EDX) spectroscopy for the analysis of elemental composition of the samples (BRUKER Nano, GmbH, D-12487). High resolution—Transmission electron microscope (HR-TEM) morphology reveals the morphology and particle size of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer using JEOL-2100 + HR-TEM. Thermogravimetric Analysis (TGA) was performed for the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer to determine the thermal stability of the nanostructures. Brunauer–Emmett–Teller (BET) analysis was carried out for the $CaCO_3$ nanofertilizer to find out the pore radius and surface area of the sample using BET surface area analyzer, Quantachrome TouchWin v1 0.2 2.

Evaluation of antifungal potential of nanofertilizer

All the glass wares were sterilized at appropriate autoclaving condition to avoid the contaminant factors. For the antifungal evaluation *Fusarium oxysporum f. sp. Lycopersici* (MTCC 10270) were stored in the PDA (potato dextrose agar) slants at 4 °C until usage. The fungal species *Fusarium oxysporum f. sp. Lycopersici* (*FOL*) were the specific causative for the vascular wilt disorder in the tomato crops affecting the growth and yield of the tomato fruits. Hence, the antifungal potential of the fertilizer mixture loaded in the $CaCO_3$ nanocarrier was assessed against *FOL* fungal culture through agar well diffusion method. For the antifungal evaluation *FOL* fungal culture were swabbed over the PDA plates using sterile cotton swabs. The *FOL* swabbed PDA plates were punctured for creating wells in the diameter of 6 mm, which was loaded with 100 µL of panchakavya extract, azadirachtin emulsion, $CaCO_3$ nanoparticles (1 mg/mL) and $CaCO_3$ nanofertilizer (1 mg/mL) along with the positive control mycostatin (1 mg/mL). Further, the above plates were incubated for 48 h at 30 ± 2 °C and after the incubation the zone of clearance with respect to the inhibition of the fungal spores and mycelia was measured and tabulated[24].

Treatment of nanofertilizer on tomato crops

Grow bags and seedlings

The purpose of the study is to enhance production and protection of the traditional tropical vegetables using nanoformulated organic fertilizer as an eco-friendly and non-toxic approach. For that, the non-germinated traditional seeds of cherry tomatoes (*Solanum lycopersicum*) were obtained from the Farmer's Council Centre, Thalayampallam Village, Tiruvannamalai, Tamilnadu, India. For the seedling and entire cultivation of the tomatoes in grow bags the pot mixture composed of 2: 2: 1: 1 ratio of sand, garden soil, coco-peat and vermicompost were used. Microbial entities in the pot mixture except vermicompost was inactivated by autoclaving at 121 °C for 30 min. The seeds were grown in the seed tray for 28 days and then it was transferred in to grow bags filled with pot mixture until the yield of tomatoes. International, national, and/or institutional norms are followed when using plants in any of the studies conducted on plants or plant parts in this study.

Foliar treatment of nanofertilizer on tomato crops

As per the standard procedure, triplicates of the tomato plantlets were treated with $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion and $CaCO_3$ nanoparticles individually using the foliar approach[25]. In the foliar approach, 10 ppm of the samples were sprayed for the first 28 days and then transferred to the grow bags the sample concentration was increased onto 30 ppm up to the yield. Herein, the $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion and $CaCO_3$ nanofertilizer were sprayed with the plantlets for once a week from the seedling to the yield of tomato fruits.

Observation of plant growth, yield and bioactive compounds with respect to nanofertilizer treatment

The significance of the samples including $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion and $CaCO_3$ nanofertilizer on the increased growth, yield and biomass of the plants towards the utilization of nutritional benefits were observed and recorded. Initially, the plant growth with number and length of the leaves, root length, stem length and height of the entire plantlet with respect to the foliar treatment of each sample were recorded. After the cultivation of the tomatoes the fresh weight and dry weight of the entire plant were measured[26]. The elemental composition from the entire plant yield was determined using the ICP-OES analysis.

Comparative evaluation of fruit yield with GCMS analysis and antioxidant assay

The weight of the tomatoes harvested on 95th day, were comparatively observed with respect to the tomatoes obtained by foliar treatment of $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion and $CaCO_3$ nanofertilizer. Furthermore, the bioactive compounds present in the fruits of tomato from the treated and control was compared using GCMS analysis. For the GCMS analysis 1 g of the homogenized fruits were blended from each treatment group and then extracted using methanol and same extract was subjected to the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radicals scavenging to assess the antioxidant potential of the samples[27].

Estimation of Lycopene and β-carotene from fruit yield

The carotenoid compound found in the tomato extract such as lycopene and β-carotene were responsible for the antioxidant potential. The lycopene and β-carotene concentration were measured in relation to the foliar application of $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion, and $CaCO_3$ nanofertilizer in order to support the antioxidant behavior of the tomato extracts. For the estimation of lycopene and β-carotene, methanol extraction of tomatoes was prepared as per the methodology of Kumari et al.[28] with some modifications as follows. About 10 g of freeze-dried tomato blend was suspended in 20 mL of methanol and was further agitated well in a rotatory orbital shaker for 2 h at 150 rpm. After the agitation, the aqueous phase from the above suspension was extracted twice with 20 mL of methanol. Afterwards, the residue was obtained by subjecting the methanol to evaporation at 37 °C for 2 days and stored at 4 °C.

The total lycopene and β-carotene content in the tomato extracts was determined using the methodology of Akter et al.[26]. The aforementioned extract was suspended in a 6:4 hexane–acetone mixture at a ratio of 10:1 (w/v) and was filtered to collect the aqueous phase. The maximum absorbance of the above aqueous extract at 663, 505, and 453 nm was measured using UV–visible spectrophotometer. The total lycopene and β-carotene contents were calculated using the formula 6 and 7 respectively as follows

$$\text{Total Lycopene content}\left(\text{mg}/100\,\text{mL}\right) = -0.0458 \times \text{Abs663} + 0.372 \times \text{Abs505} - 0.0806 \times \text{Abs453} \quad (6)$$

$$\text{Total } \beta \text{ - carotene content}\left(\text{mg}/100\,\text{mL}\right) = 0.216 \times \text{Abs663} - 0.304 \times \text{Abs505} + 0.452 \times \text{Abs453} \quad (7)$$

The absorbance values at 663, 505, and 453 nm are represented by Abs663, Abs505, and Abs453. The outcome of the absorbance value interpretation is the milligrams of β-carotene and lycopene per 100 g[29].

Toxicity evaluations of the $CaCO_3$ nanofertilizer

The institutional animal ethics committee (IAEC) of Alagappa University, Tamilnadu, India, approved the *in-vivo* toxicity assessments on freshwater zebrafish (*Danio rerio*) under the permission number AU/IAEC/Nov22/20. *In-vivo* toxicity studies was carried out using zebrafish as model system as its express sensitivity to test compounds similar to animal models based on the guidelines of OCED (Organisation for Economic Co-operation and Development) for the chemical substances, FDA (Food and Drug Administration), ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) for the pharmaceutical/food products and The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0[30,31]. Zebrafish embryo toxicity (ZET) studies is an alternative approach for the acute toxicity evaluations of drugs other than animal models. For the ZET evaluation of $CaCO_3$ nanofertilizer, the eggs of 6 hpf (hours of post fertilization) were transferred into the polystyrene plates and treated with five-fold concentrations of nanofertilizer from 50 µg/mL to 250 µg/mL. Each concentration of the nanofertilizer was treated with 20 counts of zebrafish embryos with 5 hpf maintained in the HE3 (HEPES buffered E3) media and the development of embryo was observed every 24 h up to 96 hpf using LYNX—Lawrence and Mayo phase contrast inverted microscope[32,33]. For the adult toxicity evaluation, approximately equal sized zebra fish with uniform weight and length were kept for the exposure toxicity evaluation. Two groups with ten zebra fish were adapted for the untreated control and treatment with $CaCO_3$ nanofertilizer (100 mg/L) with appropriate aeration for 5 days and the survival rate of fish was calculated. The control and treated group of zebra fish were removed from the setup and then euthanized with hypothermia (0–4 °C). Further, histopathology studies were performed with H&E (Hematoxylin and eosin) staining. All experiments were carried out in compliance with all relevant guidelines and regulations.

Statistical analysis

All the experimental values were expressed with mean ± standard deviation by performing the triplicates of each experiment using the IBM SPSS Statistics 23 software. Comparative analysis between the control and treated groups were done with Dunnett's–ANOVA test software. The Probit software (Probit Software LTD, New York, United states) was used to calculate the IC_{50} value of the experiments.

Results and discussion

Mechanism behind the fabrication of the nanofertilizer

The calcite $CaCO_3$ nanoparticles were produced by the step wise synthesis with the observable progress of impurities removal and particle size reduction[19]. The fertilizer compounds of panchakavya extract and azadirachtin emulsion were loaded within the pore sites of the $CaCO_3$ nanostructure during the agitation process of adsorption kinetics. The extraction of available nutrients and bioactive compounds from the panchakavya was confirmed by the UV–visible spectra of the panchakavya, which showed similar spectral absorbance of raw panchakavya at 301 nm (Fig. 1A). The unsaponifiable matters present in the panchakavya remains the specific absorbance at around 301 nm, which was similarly observed after the crude and vacuum filtered extract of the panchakavya[34]. The combination of hydrophilic panchakavya extract and hydrophobic azadirachtin emulsion in the $CaCO_3$ nanoparticles was performed in order to optimize the solubility of the nanofertilizer. The fertilizer loading efficiency was found to be 81.4% for the $CaCO_3$ nanofertilizer, which confirms the ability of the $CaCO_3$ nanoparticles

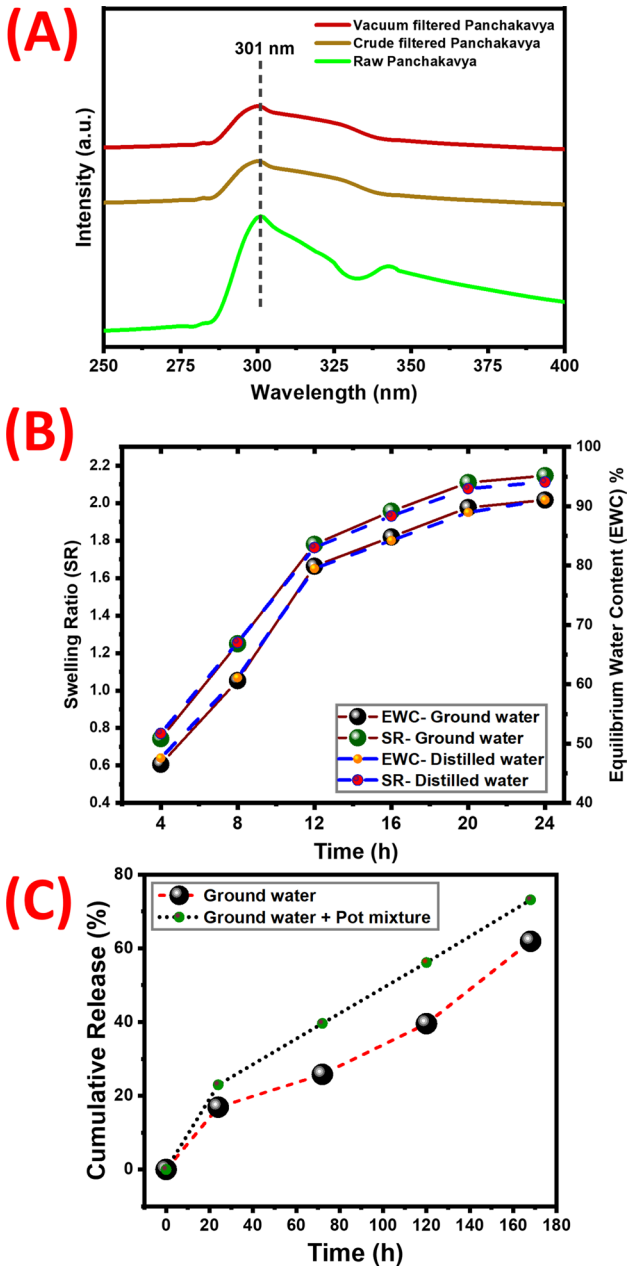


Figure 1. (**A**) UV–visible spectra raw, crude filtered and vacuum filtered panchakavya extract, (**B**) swelling ratio and Equilibrium water content of the $CaCO_3$ nanofertilizer in ground water and ground water with pot mixture and (**C**) fertilizer release profile of the $CaCO_3$ nanofertilizer ground water and ground water with pot mixture.

as a carrier system for the organic fertilizers panchakavya extract and azadirachtin emulsion. Also, here the azadirachtin was dissolved using the 1% of citronellyl acetate as a non-toxic solubilizing agent.

Physical parameter evaluation

Swelling and water equilibrium content

Swelling ratio of the $CaCO_3$ nanofertilizer was observed as 0.6 and 0.74 respectively in the distilled water and ground water during the first 4 h with equilibrium water content to be 47% and 57% respectively. The maximum swelling ratio of the nanofertilizer was observed to be 2 and 2.1 with corresponding water equilibrium content 91% and 94% respectively in distilled water and ground water after 24 h. The swelling ratio was notably higher in the ground water, when compared to the distilled water as shown in Fig. 1B. Swelling is an essential kinetics for the nanocarrier system to release the loaded content with effect to the surface erosion and disintegration of the nanoparticles. The swelling ratio and water equilibrium of the nanofertilizer witness the sustained and effective release of the fertilizer from the $CaCO_3$ nanoparticles.

Water adsorption capacity

Water adsorption is an essential phenomenon of the nanocarrier system denoted by the efficiency of adsorption of the moisture content. The water adsorption capacity of the $CaCO_3$ nanofertilizer was observed as 74% after 7 days, which indicates $CaCO_3$ nanoparticles as an efficient nanocarrier system with enhanced persistence to hold the water content and nutrients toward the utilization of the plants. The enhanced water adsorption capacity of the nanocarrier is attained due to the porous structure of the $CaCO_3$ nanoparticles, which makes it suitable candidate to serve as an excellent nanofertilizer for the plants.

Fertilizer release profile

The release profile of fertilizer mixture was evaluated at 300 nm the absorbance maxima wavelength of panchakavya and azadirachtin using UV visible spectrometer. The percentage release was calculated by the slope value concentrations of the supernatant collected from the release models with the standard calibration graph of the fertilizer mixture.

In this work, the percentage of fertilizer release was observed to be 17% and 23% respectively in the ground water and ground water with pot mixture during the first 24 h. Subsequently the percentage of fertilizer release showed a significant increase in every 48 h up to one week. Highest fertilizer release percentage was observed after seventh day as 62% and 73% in the ground water and ground water with pot mixture respectively. The fertilizer release kinetics was greatly increased in ground water with pot mixture when compared to ground water alone. From the above fact, it is clear that nanofertilizer exhibits increased adsorption of moisture content available in the pot mixture leading to enhanced swelling followed by surface erosion of the nanocarrier system thereby provoking fertilizer release. Figure 1C demonstrates the gradual fertilizer release profile of the $CaCO_3$ nanofertilizer in the ground water and pot mixture containing ground water, which shows slow and sustained release of the fertilizer up to 7 days from the nanocarrier system indicating it as a suitable nanofertilizer for the plant cultivation with the prolonged potential at lowest concentration.

Spectroscopic and material characterizations of the samples

UV–visible spectra

The UV–visible spectra of $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion and $CaCO_3$ nanofertilizer were shown in the Fig. 2A. The specific absorbance of panchakavya extract was observed at 301 nm as already justified above in the extraction part. Here, the absorbance of the calcite $CaCO_3$ nanoparticles was found at around 268 nm which was similar to the absorbance of $CaCO_3$ nanoparticles reported in the previous work[19]. Azadirachtin emulsion alone exhibits specific absorbance at around 195 nm and 298 nm, which nearly matched with the absorbance of azadirachtin compounds from the previous works[35,36]. The $CaCO_3$ nanofertilizer showed absorbance maximum at around 197 nm and 302 nm, which corresponds to the panchakavya extract and azadirachtin emulsion incorporated within the $CaCO_3$ nanoparticles. The wavelength absorbance of panchakavya extract and azadirachtin emulsion was collaboratively observed in the UV–visible spectra of nanofertilizer along with a reduction of peak intensity; which ensures the loading of fertilizer mixture in the $CaCO_3$ nanocarrier system. Both panchakavya and azadirachtin compounds showed maximum absorbance at around 300 nm, hence loading and release profiles of the fertilizer mixture consisting of panchakavya and azadirachtin was observed at 300 nm.

FTIR spectra

The FTIR spectra of $CaCO_3$ nanoparticles, panchakavya extract, azadirachtin emulsion and $CaCO_3$ nanofertilizer were shown in Fig. 2B. The $CaCO_3$ nanoparticles showed functional transmittance peaks at 711, 870 and 1453 cm^{-1} corresponding to the symmetrical and asymmetrical stretching vibrations of carbonate groups (CO_2^{3-}) present in the calcite $CaCO_3$[19,37]. Panchakavya extract showed the functional frequency peaks at 1448 cm^{-1} for the C–C stretching of aromatic compounds and 3389 cm^{-1} for the O–H stretching of phenols and alcohols. The remaining peaks of panchakavya at 2335, 2111 and 1634 cm^{-1} specified the presence of primary amines and alkanes present in the extract[12,38]. The broad peak of azadirachtin emulsion around 3312 cm^{-1} indicates the bending and stretching vibrations of amino (N–H) groups and the functional frequencies of aliphatic C–H stretch was observed at 2922 and 2848 cm^{-1}. Also, the azadirachtin was observed with functional frequencies at 669 cm^{-1} for the rocking vibrations of methylene group, 1098 cm^{-1} for the C–O–C stretching vibrations of ethers and 1638 cm^{-1} for the carboxyl (C=O) group in accordance to the previous reports on azadirachtin emulsion[39,40]. In the FTIR spectra of the $CaCO_3$ nanofertilizer, exhibited peaks at 714 and 879 cm^{-1} representing

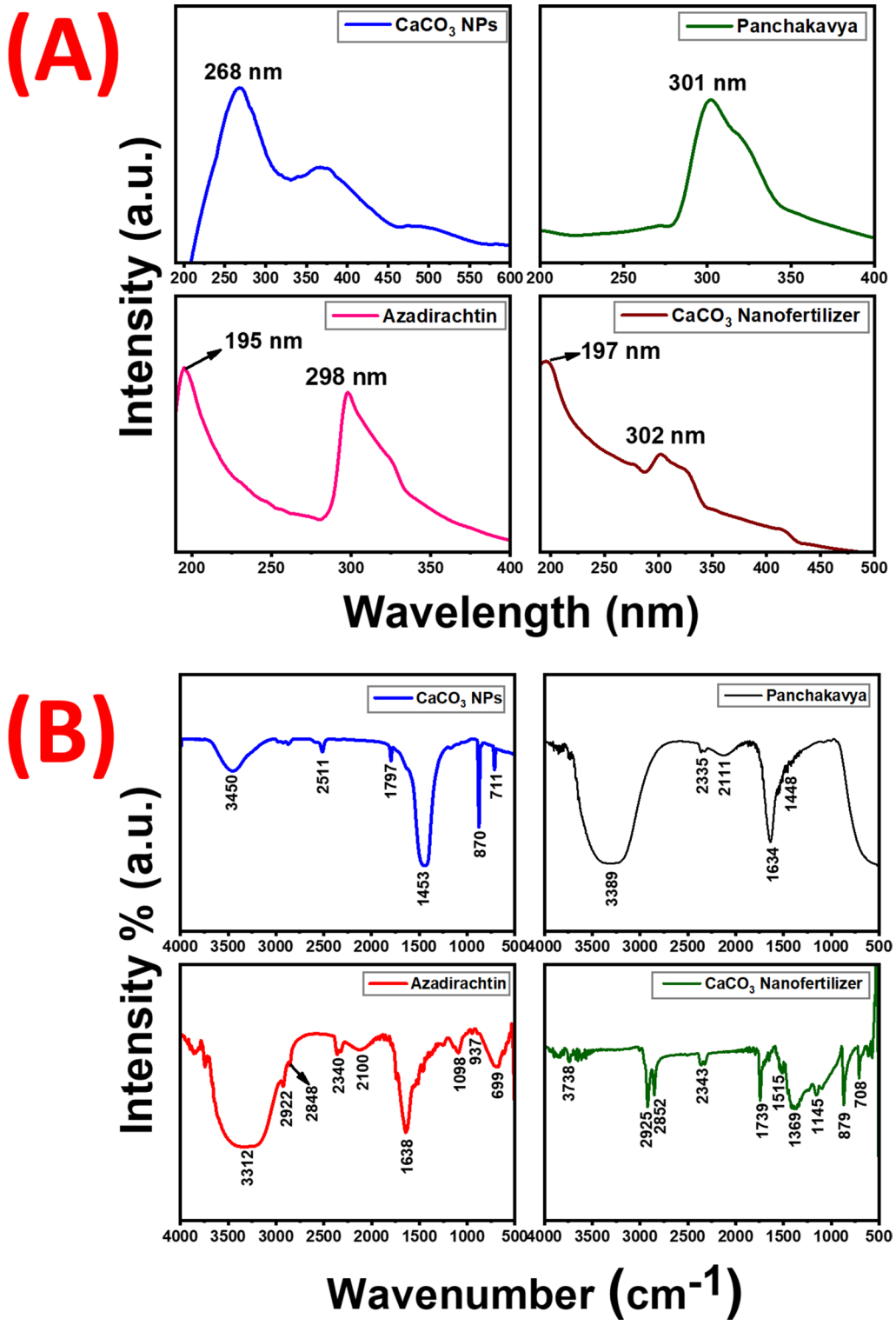


Figure 2. (**A**) UV–visible and (**B**) FTIR spectra of $CaCO_3$ nanoparticles, Panchakavya extract, Azadirachtin emulsion and $CaCO_3$ nanofertilizer.

the stretching vibrations of CO_2^{3-} groups of $CaCO_3$ nanoparticles. FTIR peaks at 2925 and 2852 cm^{-1} observed in nanofertilizers represents the aliphatic C–H stretching of the azadirachtin and 2343 cm^{-1} corresponds to the primary amines and alkanes of the panchakavya extract. FTIR peaks of $CaCO_3$ nanofertilizer matched with the $CaCO_3$ nanoparticles, panchakavya emulsion and azadirachtin with slight red shift due the incorporation of the fertilizer mixture with nanocarrier system.

XRD pattern

The crystalline structure of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer was determined by the X-ray diffraction studies (Fig. 3A). The XRD pattern of the $CaCO_3$ nanoparticles synthesized from the conch shell waste retains the calcite polymorph of the $CaCO_3$ which matched well with the ICDD reference no. 00-005-0586. The conch waste derived $CaCO_3$ nanoparticles exhibits miller indices planes of (0 1 2), (1 0 4), (0 0 6), (110), (113), (202), (024), (0 1 8), (2 1 1), (3 0 0) and (0 0 12) corresponding to 2θ values of 22°, 28.8°, 31°, 35°, 38.8°,

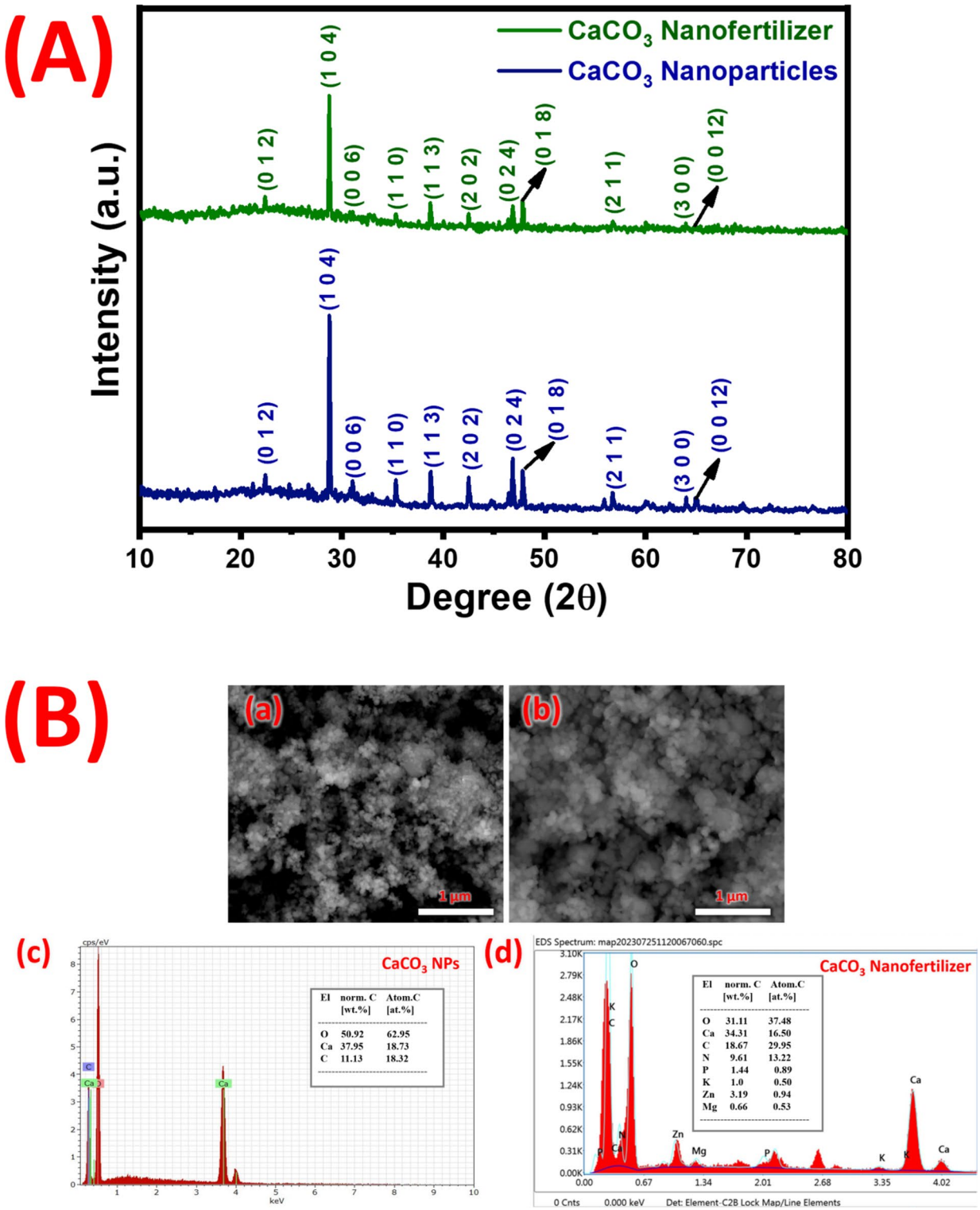


Figure 3. (**A**) XRD pattern of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer and (**B**) FE-SEM morphology of (**a**) $CaCO_3$ nanoparticles, (**b**) $CaCO_3$ nanofertilizer and EDX spectra of (**c**) $CaCO_3$ nanoparticles and (**d**) $CaCO_3$ nanofertilizer.

42°, 47.07°, 47.8°, 56°, 64° and 65° indicating rhombohedral calcite crystal structure[19]. The $CaCO_3$ nanofertilizer exhibits the same crystalline structure of the calcite $CaCO_3$ nanoparticles with similar miller indices planes and 2θ values. The panchakavya extract contains organic and inorganic nutrients like potassium (K), phosphorous (P), zinc (Zn), magnesium (Mg), which are available in the minor ionic phase, hence these minerals will not be assessed by X-ray diffraction as XRD pattern determines mostly the crystalline structure of the predominant material present in the sample like $CaCO_3$ in the nanofertilizer. Reduction in peak intensity in nanocomposites might be due to the addition of the fertilizer mixture within the $CaCO_3$ nanoparticles; hence loading of the fertilizer mixture doesn't affect the crystalline structure of the $CaCO_3$ nanoparticles. The calcite crystalline structure of $CaCO_3$ nanoparticles determined by the XRD pattern, ensures that the material stability of nanofertilizers is appropriate for the prolonged release of fertilizer mixture in crop cultivation environment. Because, calcite is the most stable form of $CaCO_3$ when compared to vaterite and aragonite, which are other crystalline forms of $CaCO_3$.

FE-SEM and EDX

The morphology of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer in the FE-SEM images were shown in Fig. 3B (a&b). The morphology of the $CaCO_3$ nanoparticles was observed as quasi spherical shaped particles with scattered arrangement as reported in our previous work[19]. On other hand, $CaCO_3$ nanofertilizer showed slightly integrated units of $CaCO_3$ nanoparticles, which might be due the incorporation of the panchakavya extract and azadirachtin emulsion with the nanostructure. Anyhow, the morphology of the $CaCO_3$ nanoparticles was not deformed in the nanofertilizer as represented in the XRD pattern of the nanofertilizer. The EDX spectra of $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer clearly illustrates the elemental composition the samples displayed in the Fig. 3B (c&d). The $CaCO_3$ nanoparticles exhibit elemental composition of calcium (Ca), carbon (C) and oxygen (O) respectively, with the weight percentage of 37.95%, 11.13% and 50.92% respectively. Herein, the elemental composition of the $CaCO_3$ nanoparticles ensures the yield of calcite $CaCO_3$ from the conch shell waste by the elimination of impurities through reinforced synthesis procedure[19]. Along with Ca (24.35%), C (24.46%) and O (50.36%), the $CaCO_3$ nanofertilizer shows elemental composition of sodium (Na) and phosphorous (P) with the weight percentage of 0.47% and 0.38% respectively. In this case the elemental addition of Na and P were implied from the panchakavya extract to the $CaCO_3$ nanofertilizer; overall, the major composition of nanofertilizer comprises of Ca, C and O respectively. Here, the elemental composition of the nanofertilizer indicates the presence of essential primary nutrients N, P, K along with calcium which are sufficient for the upscaled growth and yield of the crops[41].

DLS and zeta potential

Average particle of the nanostructures was evaluated by the DLS spectra based on the Brownian motion of the particles in the solution and the results were showed in Fig. 4A (a&b). The average particle size of the $CaCO_3$ nanoparticles was found to be 61 nm in diameter, which closely related to the particle size of conch waste derived $CaCO_3$ nanoparticles obtained from our previous work (59.2 nm). From our previous work, it is confirmed that impurity elements from conch shell waste were significantly removed and particle size of the $CaCO_3$ was reduced into the scale of nanometer by the four phases of synthesis procedure[19]. The average particle size of the $CaCO_3$ nanofertilizer was observed to be 89 nm, which is higher than the particle size of the $CaCO_3$ nanoparticles alone. Increased particle size of nanofertilizer might be due to loading of fertilizer mixture with $CaCO_3$ nanoparticles. However, the particle sizes up to 100 nm can penetrate in to the plants through foliar treatment and the particle size up to 500 nm can transverse the cell membrane of the fungal organisms[25,42]. The polydisperse index (PDI) value of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer was noticed to be 0.431 and 0.381 respectively, which indicates that the nanostructure exhibits mid-range polydispersity. The zeta potential charge of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer was observed to be − 26.2 mV and − 23 mV respectively (Fig. 4A (c&d)) indicating the optimum stability of the prepared nanostructures. Results conclude that size, dispersity and surface charge of the $CaCO_3$ nanoparticles is suitable for the antifungal assessment and foliar treatment for the plant production.

HR-TEM

The TEM morphology of the $CaCO_3$ nanoparticles showed randomly distributed particles of $CaCO_3$ nanoparticles with the nearly cubic and quasi spherical shapes (Fig. 4B (a)). The well-confined morphology was achieved through the effect of four phases of the synthesis protocols. The repeated progress of the drying and calcination tuned the particles purity level and reduced particles size leading to the yield of calcite $CaCO_3$ nanoparticles. The morphology of $CaCO_3$ nanofertilizer was observed as quasi spherical and cubic shaped particles with increased particle size due to loading fertilizer compound with the $CaCO_3$ nanoparticles (Fig. 4B (b)). The average particle size calculated from the HR-TEM images for $CaCO_3$ nanoparticles alone and $CaCO_3$ nanofertilizer using ImageJ software was observed to be 32 and 40 nm respectively. Since the particle size of nanofertilizer is < 500 nm it can readily transverse the plant and microbial cells effectively playing the role of active fertilizer and fungicide. In addition, the structure of the $CaCO_3$ nanoparticles was not affected by loading of fertilizer as per the results of XRD and FE-SEM Analysis.

TGA analysis

The thermal stability of the $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer were assessed using thermo-gravimetric analysis and the results are displayed in Fig. 5A. TGA curve shows no drastic weight loss in the $CaCO_3$ nanoparticles alone up to 800 °C, which indicates the greater thermal stability of the calcite $CaCO_3$. $CaCO_3$ nanofertilizer showed 3 phases of weight loss at 25 to 330 °C, 340 to 600 °C and 610 to 780 °C respectively with the weight loss percentage of 10%, 2% and 40%. Initial weight loss of the $CaCO_3$ nanofertilizer at 250 °C may

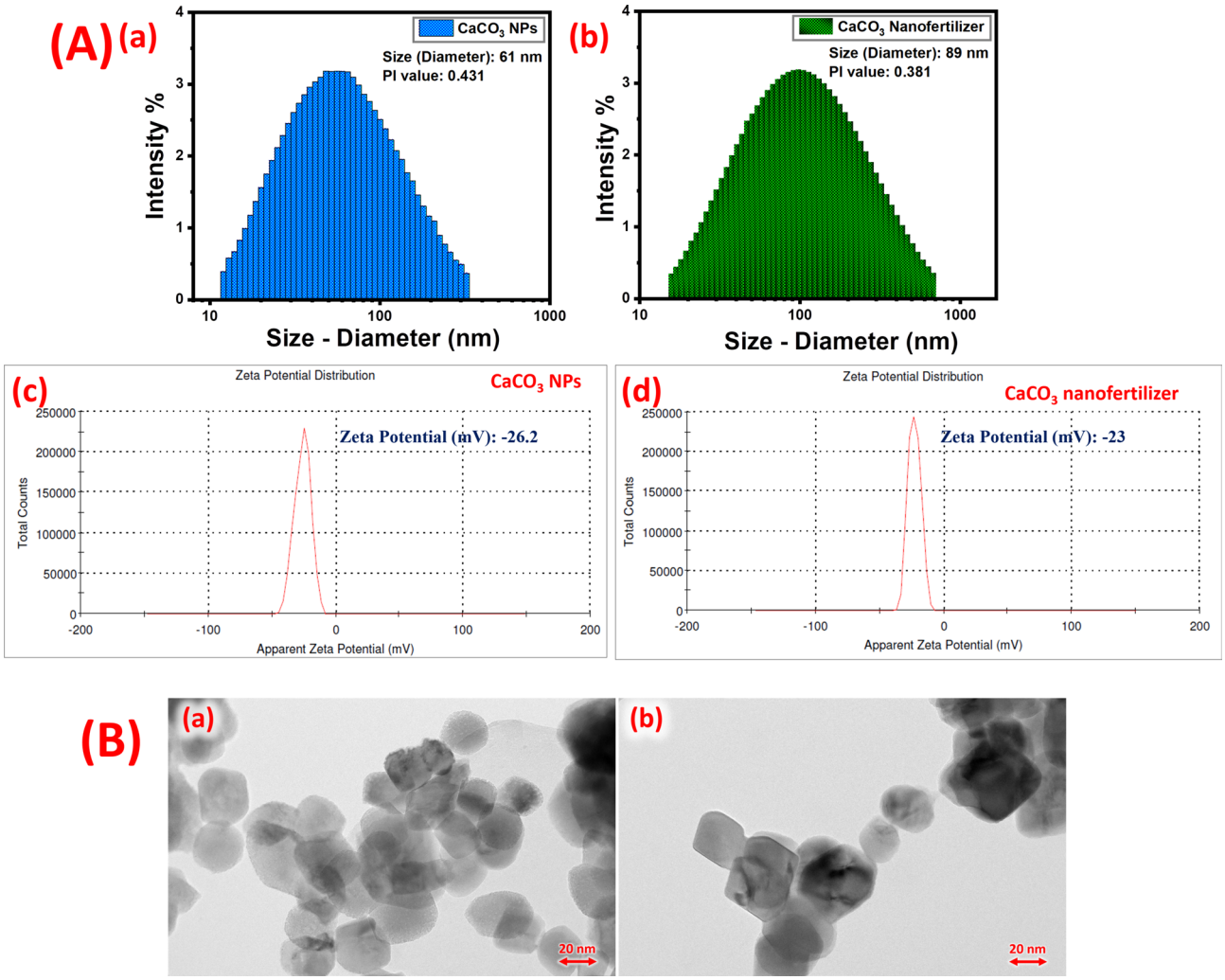


Figure 4. (**A**) DLS spectra of (**a**) $CaCO_3$ nanoparticles, (**b**) $CaCO_3$ nanofertilizer and Zeta potential evaluation of (**c**) $CaCO_3$ nanoparticles and (**d**) $CaCO_3$ nanofertilizer and (**B**) TEM micrograph of (**a**) $CaCO_3$ nanoparticles and (**b**) $CaCO_3$ nanofertilizer.

attributed to the evaporation of the moisture content, while the remaining phases of weight loss reveals the decomposition of the residual organic maters present in the fertilizer (panchakavya extract and azadirachtin emulsion) mixture[43].

BET analysis

BET isotherm analysis of the $CaCO_3$ nanofertilizer reveals the pore radius, pore volume, surface area and the isotherms of nitrogen adsorption and desorption (Fig. 5B). The surface area of the $CaCO_3$ nanofertilizer was observed to be 24.879 m^2/g and the pore radius in diameter were 4.0239 nm with the pore volume of 5.005 $6cm^3/g$ which infers that fabricated nanofertilizer architects with mesoporous structure[44]. Adsorption and desorption plots of the $CaCO_3$nanofertilizer belongs to the type III isotherm of the mesoporous material[45]. The mesoporous nanostructures were considered as a factor for enhancing the water retention capacity in the soil and solid–liquid interactions significantly, which is useful for the enhanced plant growth[46,47].

Antifungal potential of the $CaCO_3$ nanofertilizer

Figure 6A showed the antifungal evaluation of panchakavya, azadirachtin emulsion, $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer along with control (loaded with millipore water) positive control (loaded with mycostatin). The control plate was fully covered with fungal spores and mycelia of *FOL*, while positive control mycostatin showed zone of inhibition of 38 mm against *FOL* in well diffusion method. The $CaCO_3$ nanofertilizer showed highest potential against *FOL* with zone of inhibition of 41 mm when compared to the $CaCO_3$ nanoparticles (11 mm), panchakavya extract (28 mm) and azadirachtin emulsion (35 mm) alone respectively (Table 1). Zone of clearance in all the samples were slightly observed due to the interruption of overgrown fungal mycelia of *FOL*, which fall over the non-inhibited circumference of the PDA plates. Overall, the synergistic potential of $CaCO_3$ nanoparticles, panchakavya extract and azadirachtin emulsion in the $CaCO_3$ nanofertilizer exhibits excellent

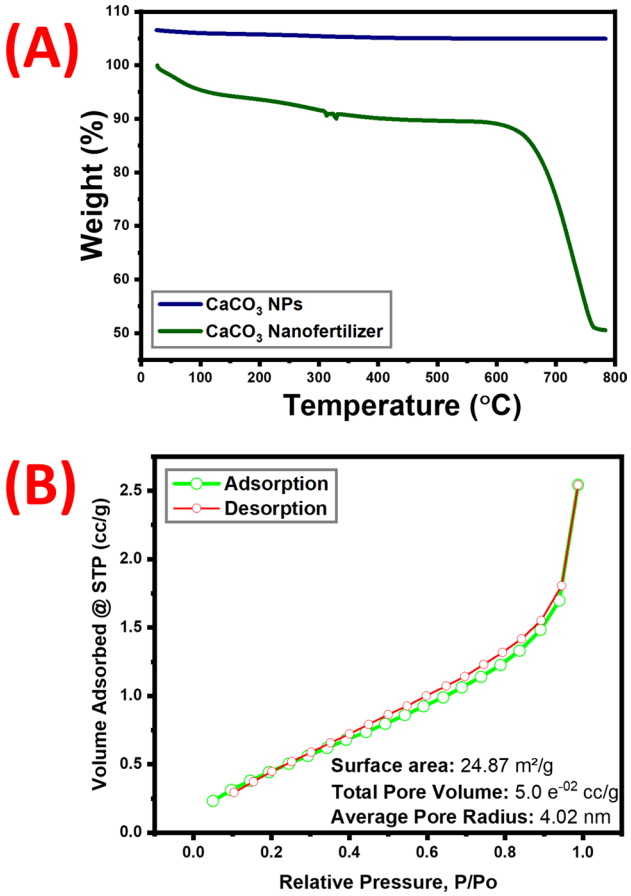


Figure 5. (**A**) TGA analysis of $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer and (**B**) BET adsorption and desorption isotherm of $CaCO_3$ nanofertilizer.

antifungal activity against *FOL*. Thus, nanofertilizer fabricated through the formulation of organic fertilizer with nanocarrier system helps to control and treat the fusarium wilt disorders in the plants.

Plant growth and fruit yield observations after $CaCO_3$ nanofertilizer

Plantlet observation at 14th day

The plantlet observation after 14th day from the seedling exposed to two passages of the foliar treatment of the samples including $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer were shown in Fig. 6B. The growth difference in the triplicates of each sample treatment was observed with plantlet height (Fig. 7A), number of leaves (Fig. 7B), length of the stem and root (Fig. 7C). The bar diagrams reveal the growth observation of $CaCO_3$ nanofertilizer treated groups which indicates enhanced growth rate with respect to height, number of leaves, shoot and root length, when compared to the blank group. Fertilizer compounds alone treated groups showed remarkable growth rate, when compared to the $CaCO_3$ nanoparticles alone; however, $CaCO_3$ nanofertilizer showed increased growth rate due to synergistic effect of the nanoparticles and fertilizer mixture resulting in higher growth rate in the plantlets.

Entire plant observation after yield

The plant observation was done with the fresh weight and dry weight of the entire tomato plant except fruits after the 95th day of cultivation (Fig. 8A). The wet and dry weight of the plants based on the biomass production of the plants was observed to be 632 ± 3.785 g of fresh weight and 169 ± 2.645 g of dry weight for $CaCO_3$ nanofertilizer treated plants when compared to the $CaCO_3$ nanoparticles (560 ± 2.516 g fresh and 146 ± 1.527 g dry), panchakavya extract (589 ± 2.516 g fresh and 166 ± 2.516 g dry), azadirachtin emulsion (539 ± 2.645 g fresh and 141 ± 1.527) along with the un-treated control (519 ± 3.214 g fresh and 137 ± 2.081). After the observation the fresh and dry weight of tomato plants, each part of the dried plants including stem, root, leaves and fruits were collected at equal ratio of weight and digested with nitric acid for ICP-OES analysis to predict the calcium (Ca), phosphorus (P), potassium (K), zinc (Zn) and magnesium (Mg) elements with effect to the foliar treatment. The ICP-OES analysis results infers that the plant treated with $CaCO_3$ nanofertilizer showed enhanced nutritional value for the Ca, P, K, Zn and Mg around 4.235, 2.177, 4.92, 2.315 and 1.425 µg/ml respectively, when compared to the other treatments and control as tabulated in Table 2. Here, the obtained values of ICP-OES analysis were matched with the certified reference material (CRM-NCS ZC85006 Tomato) from National Analysis Centre,

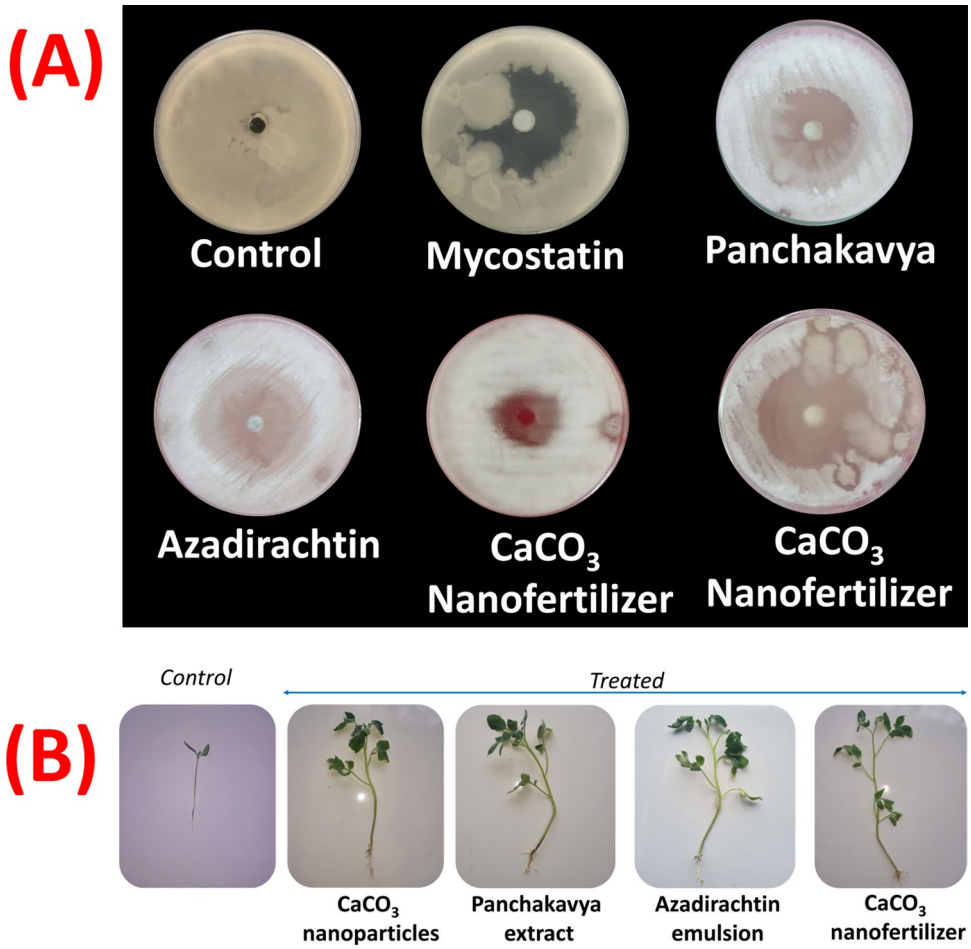


Figure 6. (**A**) Antifungal evaluation of $CaCO_3$ nanoparticles, Panchakavya extract, Azadirachtin emulsion and $CaCO_3$ nanofertilizer against *Fusarium oxysporum f. sp. Lycopersici* and (**B**) Plantlet observation with effect to the foliar treatment of $CaCO_3$ nanoparticles, Panchakavya extract, Azadirachtin emulsion and $CaCO_3$ nanofertilizer.

S. no	Samples	Loading volume	Zone of inhibition (mm)
1	Control (Millipore water)		Nil
2	Positive control (Mycostatin-1 mg/mL)		38 mm
3	Panchakavya extract	100 µL	28 mm
4	Azadirachtin emulsion		35 mm
5	$CaCO_3$ nanoparticles (1 mg/mL)		11 mm
6	$CaCO_3$ nanofertilizer (1 mg/mL)		41 mm

Table 1. Antifungal evaluation against *Fusarium oxysporum f. sp. Lycopersici.*

China. The observation of plant weight and elemental composition illustrates that the foliar treatment of the $CaCO_3$ nanofertilizer enhances the production and nutritional uptake in tomato crops.

Screening of fruit yield and its antioxidant potential

Total yield was observed from the complete ripened tomatoes collected from the 80 to 95 days of cultivation (Fig. 8B). In the non-treated control, the yield was observed to be 272 ± 2 g of tomatoes with small size and minimum quantity, while the fruit weight of foliar treatment was observed to be 767 ± 2.516 g for $CaCO_3$ nanofertilizer, 478 ± 3.214 g for $CaCO_3$ nanoparticles, 355 ± 8.326 g for panchakavya extract and 271 ± 6.429 g for azadirachtin emulsion, respectively. In the untreated control plants of tomatoes mealybugs pest attack was observed during the rainy season (Fig. 9A). But there was no pest and bugs attack in the plant groups treated with panchakavya, azadirachtin emulsion, $CaCO_3$ nanoparticles and $CaCO_3$ nanofertilizer. Foliar treatment of

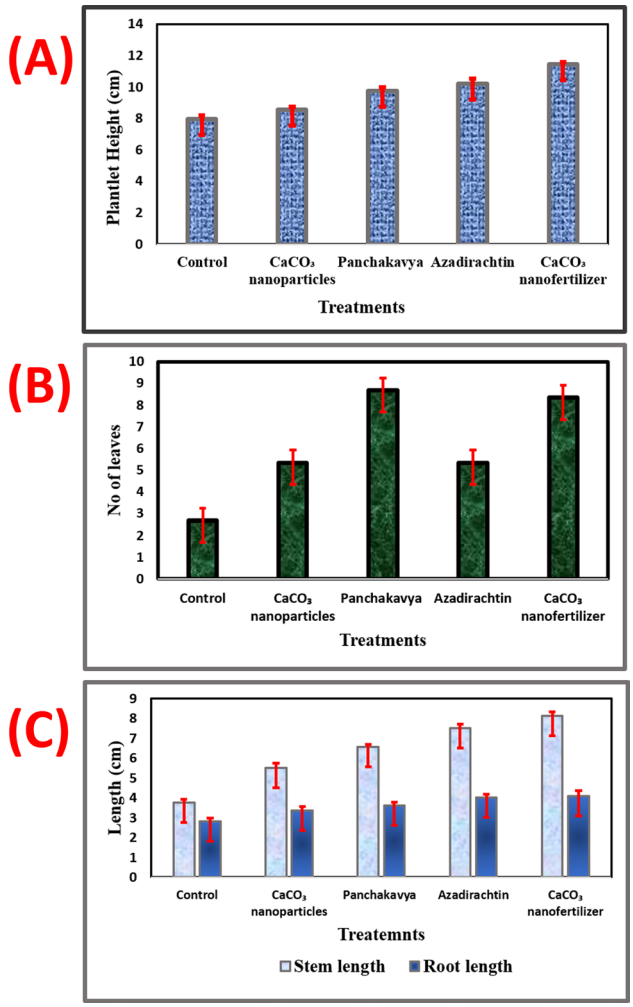


Figure 7. Observation of (**A**) Height, (**B**) no of laves and (**C**) length of the root and stem of the plantlets with effect to the foliar treatment of foliar treatment of $CaCO_3$ nanoparticles, Panchakavya extract, Azadirachtin emulsion and $CaCO_3$ nanofertilizer.

panchakavya extract and $CaCO_3$ nanoparticles showed increased size of fruits when compared to the azadirachtin emulsion and $CaCO_3$ nanofertilizer. However, the fruit yield count and weight of the tomatoes was comparatively much greater in the plant treated with $CaCO_3$ nanofertilizer than the plants treated with $CaCO_3$ nanoparticles, panchakavya extract and azadirachtin emulsion (Fig. 9B).

Antioxidant potential of methanolic extract of tomatoes treated with nanofertilizer by foliar approach was assessed by DPPH free radical scavenging assay and the results were shown in Fig. 9C. Next to the positive control BHT (Butylated hydroxytoluene), the free radical scavenging was observed as $31 \pm 1.142\%$ for 50 µL and $52 \pm 0.887\%$ for 100 µL of extracts of tomato obtained from $CaCO_3$ nanofertilizer treated plants. Free radical scavenging activity of fruit yield extract of plants treated with nanofertilizer was comparatively higher than the $CaCO_3$ nanoparticles, panchakavya extract and azadirachtin emulsion. The antioxidant potential of the tomato extract was majorly based on the lycopene and β-carotene present in the tomatoes[27,48]. Foliar treatment of the $CaCO_3$ nanofertilizer enhances the carotenoids level in the tomatoes, which will be useful to overcome the oxidative stress mediated disorders caused by the free radical accelerating systems such as reactive oxygen species (ROS).

GC–MS analysis of the fruit yield

The GC–MS analysis was carried out to determine the active organic compounds present in the tomatoes after the foliar treatment with $CaCO_3$ nanofertilizer, $CaCO_3$ nanoparticles, azadirachtin emulsion and panchakavya extract comparatively with the non-treated control. In the GC–MS spectra, tomato extract of the plant treated with $CaCO_3$ nanofertilizer was identified with maximum bioactive compounds including citronellyl propionate (6-Octen-1-ol, 3,7-dimethyl-, propanoate), oleic acid and vitamin E (alpha.-tocopherol), respectively. Percentage of retention area were lesser in the tomato extract from the $CaCO_3$ nanofertilizer treated plants, when compared to the other treatment and control due to the presence of several bioactive compounds such as oleic acid when compared to other treated groups (Table 3). From the GC–MS results, it was ensured that treatment with $CaCO_3$ nanofertilizer enhances the metabolism of tomato plants towards the enhanced production of bioactive

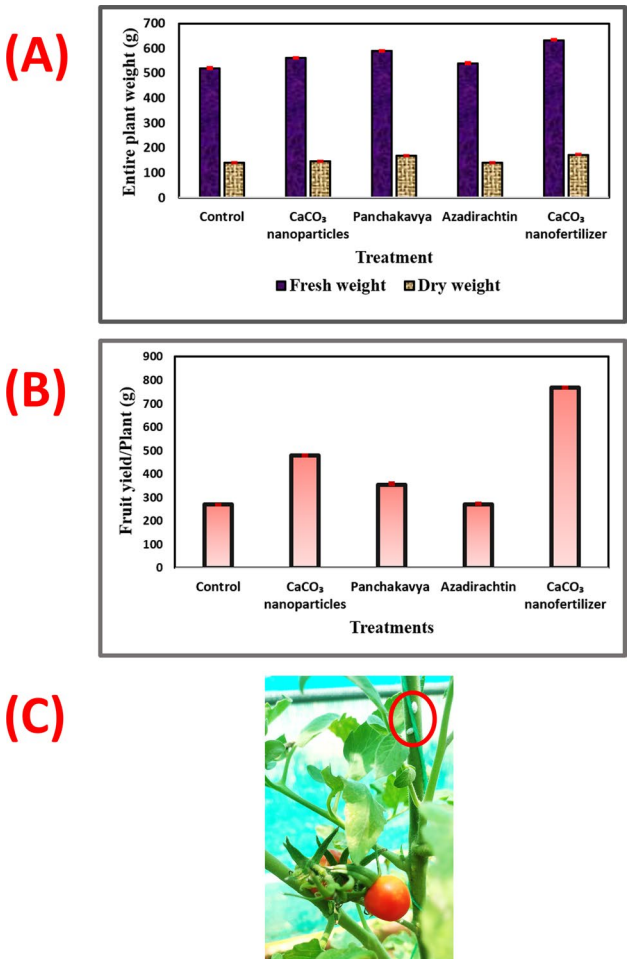


Figure 8. (**A**) Entire plant weight and (**B**) Total fruit yield from 80 to 95 days of tomato cultivation with effect to the foliar treatment of foliar treatment of $CaCO_3$ nanoparticles, Panchakavya extract, Azadirachtin emulsion and $CaCO_3$ nanofertilizer and C) Observation of Mealybugs in the non-treated control tomato plant.

S. no	Elements	Dilution factor	Concentration in ppm (µg/mL)				
			S1#	S2#	S3#	S4#	S5#
1	Calcium (Ca)		4.235	3.897	3.723	4.09	3.472
2	Phosphorus (P)		2.177	1.122	2.017	2.115	1.891
3	Potassium (K)	100	4.92	3.732	3.791	4.656	3.971
4	Zinc (Zn)		2.315	2.138	1.891	2.273	2.073
5	Magnesium (Mg)		1.425	0.982	0. 998	1.01	0.977

Table 2. ICP-OES analysis for the elemental determination of tomato plants. #Sample code S1, S2, S3, S4 and S5 denotes elemental detection from the plant yield with respect to the foliar treatment with $CaCO_3$nanofertilizer, $CaCO_3$ nanoparticles, azadirachtin emulsion, panchakavya extract and non-treated control respectively.

compounds when compared to the other samples and control. The bioactive compounds present in tomatoes, exposed to nanofertilizers significantly enhances the taste and nutritional value of the fruits.

Determination of total Lycopene and β-carotene present in the tomato extract

Lycopene is a non-oxygenated carotenoid that exhibits much greater antioxidant potential when compared to the oxygenated carotenoid i.e. β-carotene. Since, the oxygenated β-carotene is hypersensitive to light, oxygen, and temperature[49]. In this study, the lycopene content was high when compared to β-carotene in the extract of cherry tomatoes treated with fertilizer compounds. The extract of tomatoes treated with $CaCO_3$ nanofertilizer showed higher lycopene content of 0.82 ± 0.008 mg/100 g when compared to the $CaCO_3$ nanoparticles (0.61 ± 0.005 mg/100 g), panchakavya extract (0.69 ± 0.009 mg/100 g), azadirachtin emulsion

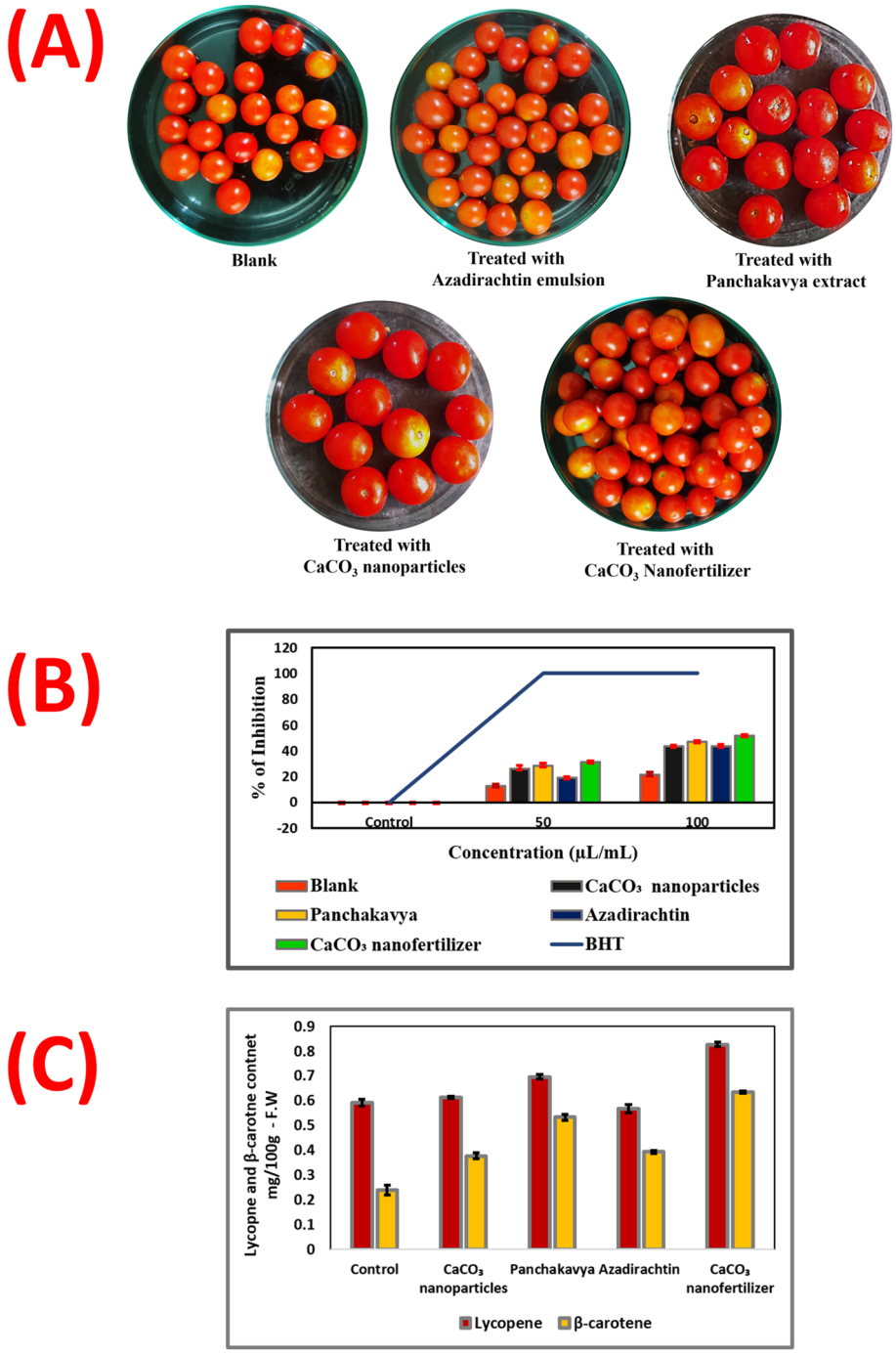


Figure 9. The effect of foliar treatment of foliar treatment of $CaCO_3$ nanoparticles, Panchakavya extract, Azadirachtin emulsion and $CaCO_3$ nanofertilizer in the (**A**) Fruit yield of tomatoes, (**B**) DPPH free radical scavenging effect of the tomato extracts and (**C**) estimation of Lycopene and β-carotene content in the tomato extracts.

(0.69 ± 0.016 mg/100 g) and untreated control (0.56 ± 0.013 mg/100 g), respectively. Total β-carotene content of $CaCO_3$ nanofertilizer treated groups was observed as 0.63 mg/100 g which is higher than $CaCO_3$ nanoparticles (0.37 ± 0.012 mg/100 g), panchakavya extract (0.53 ± 0.012 mg/100 g), azadirachtin emulsion (0.39 ± 0.006 mg/100 g) treated groups and the control group (0.23 ± mg/100 g), respectively (Fig. 9C). The increase in carotenoid content is responsible for the enhanced antioxidant potential as substantiated in the DPPH free radical scavenging assay.

S. no	Name of the compound	Molecular weight (g/mol)	Formula	Retention area %				
				S1*	S2*	S3*	S4*	S5*
1	6-Octen-1-ol, 3,7-dimethyl-, propanoate	212.33	$C_{13}H_{24}O_2$	94.05	99.76	86.65	82.49	98.22
2	3,7-Dimethyloct-6-enyl ethyl carbonate	228.33	$C_{13}H_{24}O_3$	82.12	84.21	96.12	83.84	78.21
3	6-Aminotetrazolo (b) pyridazine	1426.1	$C_4H_4N_6$	68.51	84.24			
4	Pent-2-ynal	82.1	C_5H_6O	59.99			48.65	
5	Formaldehyde, methyl(2-propynyl) hydrazone	96.13	$C_5H_8N_2$	41.82		86.17		47.89
6	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	79.38	86.77	83.93	73.99	79.38
7	trans-13-Octadecenoic acid	282.5	$C_{18}H_{34}O_2$	38.79	75.64			35.56
8	E-11-Hexadecenal	238.41	$C_{16}H_{30}O$	70.87		57.54	50.92	
9	7,11-Hexadecadienal	236.39	$C_{16}H_{28}O$	72.64	50.08		43.96	69.37
10	Pyrazole, 3-methyl-5-(3,5-dimethylpyrazol-1-yl)-4-nitro-	221.22	$C_9H_{11}N_5O_2$			51.83	61.98	
11	Dehydroabietic acid	300.4	$C_{20}H_{28}O_2$	77.65	69.08	94.86	69.37	
12	13-Octadecenal, (Z)	266.5	$C_{18}H_{34}O$	53.72	63.34		52.02	
13	Alpha.-tocopherol	430.7	$C_{29}H_{50}O_2$	58.32				61.34
14	Oleic acid	282.5	$C_{18}H_{34}O_2$	50.61		65.49		

Table 3. GC–MS analysis of tomato extracts. *Here sample codes S1, S2, S3, S4 and S5 denotes tomato extract with respect to the foliar treatment with $CaCO_3$nanofertilizer, $CaCO_3$ nanoparticles, azadirachtin emulsion, panchakavya extract and non-treated control respectively.

Mechanism behind $CaCO_3$ nanofertilizer in tomato cultivation

The perspective of the $CaCO_3$ nanofertilizer fabricated in this work is about the immobilization of the organic fertilizer with the $CaCO_3$ nanoparticles as a carrier system for sustained release of the fertilizer. The foliar spray is the short and effective approach for the enhanced nutritional uptake in plants suitable for the short-term plants such as tropical vegetables. In the foliar spray approach, the nanoparticles enter through the symplast movement interconnected by the plasmodesmata of leaves. After the entry the sustained release of the organic compound enhances the biomass production through the photosynthesis. Photosynthesis requires primary nutrients NPK, which consists of nitrogen, phosphorous and potassium along with calcium. The calcium regulates the stomatal movement and proteins of photosynthesis of plants along with enhanced nutritional uptake[50,51]. In this work, the $CaCO_3$ nanoparticles and panchakavya extract can release the calcium ions significantly to the plant sites along with the NPK and growth regulators present in the panchakavya extract. The azadirachtin in the nanofertilizer formulation was helpful for the optimized solubility and enhanced protection from the pest attack. Also, the fertilizer mixture consists of azadirachtin emulsion and panchakavya extract, which acts as effective antifungal agents significantly helpful to control and treat the plant affecting fungal organisms i.e., *FOL*[52,53]. Most importantly, the size of the $CaCO_3$ nanofertilizer suits for the permeability towards the pore sites of the plants and microorganism, implying enhanced fertilizer delivery in a controlled manner and antifungal potential against *FOL*. The non-adsorbed nanofertilizer accumulated on the pot mixture was also adsorbed by the xylem through the apoplast and symplast movements of the roots[54]. Here, the $CaCO_3$ nanocarrier system protects the organic fertilizers from the biotic and abiotic stress factors affecting the organic compounds present in the fertilizers. Each compound formulated in the nanofertilizer (including $CaCO_3$ nanoparticles, azadirachtin emulsion and panchakavya extract) were non-toxic in nature and the minimum concentration of the nanofertilizer is sufficient for the enhanced production/protection of tomato crops. Hence, this work plan fabricated the non-toxic, low cost and effective nanofertilizer for the tomato cultivation. Figure 10 is about the schematic representation of the fabrication of nanofertilizer and its action of mechanism in plant's nutritional uptake, growth and yield against biotic/abiotic stress factors.

Toxicity evaluations

Toxicity evaluation with zebrafish embryo model

The ZET evaluation of different concentrations of $CaCO_3$ nanofertilizer showed no significant malformations in the zebrafish embryo development from 24 hpf up to 96 hpf similar to control group. Even, the highest concentration at 250 µg/mL $CaCO_3$nanofertilizer showed no significant abnormalities in the organelle development of the zebrafish larvae including head, eyes, cardiac loops, chord and tail (Fig. 11) regions ensuring the biosafety level of the nanofertilizer to the human and animal exposure.

Adult toxicity studies

Exposure toxicity of the $CaCO_3$ nanoparticles was done with zebrafish (*Danio rerio*) as model system to ensure the biosafety level of the nanofertilizer in soil environment and also in human health. Approximately 70% of genetic organization of the zebrafish matched with the human genome; therefore, zebrafish was considered as better alternative to the animal models for the toxicity evaluations[55]. No significant malformation in the physical features was observed in zebrafish model system treated with $CaCO_3$ nanofertilizer (Fig. 12A). Survival rate of the zebra fish was observed to be 100% up to 5 days on exposure to nanofertilizer (Fig. 12B). Histopathological

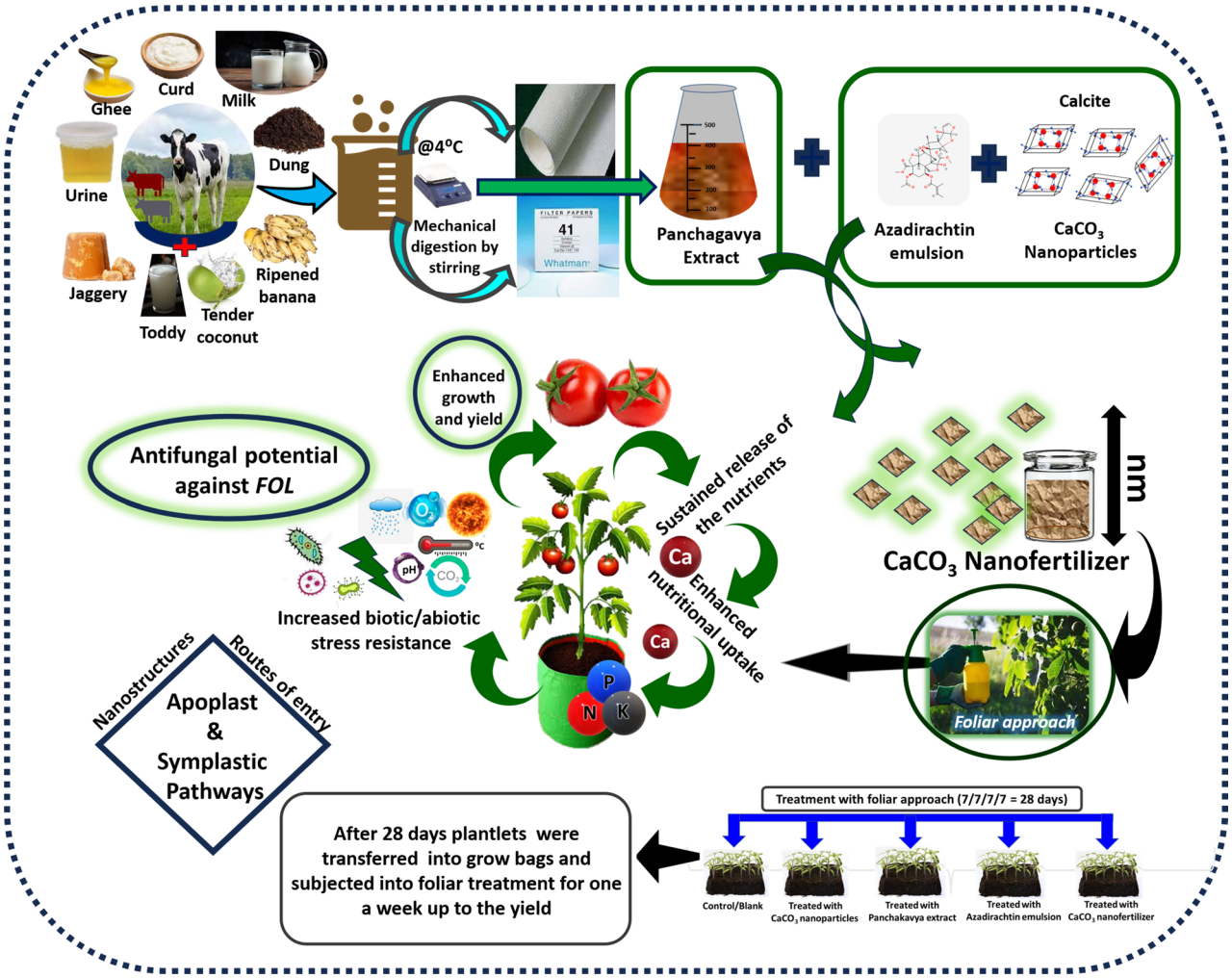


Figure 10. Schematic representation for the fabrication of nanofertilizer and its action of mechanism in plant growth and yield.

observation of organs like gill, brain, liver, intestine and kidney showed no significant changes in $CaCO_3$ nanofertilizer treated groups and was identical to control fish group (Fig. 12C). Above all, it is certified that $CaCO_3$ nanofertilizer fabricated through this work protocol was non-toxic and eco-friendly in nature.

Conclusion

The present study focused on fabrication of $CaCO_3$ nanofertilizer based on the adsorption technique, which involves the adsorption immobilization of fertilizer mixture azadirachtin emulsion and panchakavya extract within the pore sites of $CaCO_3$ nanoparticles. In $CaCO_3$ nanofertilizer, the $CaCO_3$ nanoparticles is derived from conch waste and the panchakavya extract from cow's by products representing waste management and low-cost production of nanofertilizer. According to material characterization studies that included UV–visible, FTIR spectra, and XRD patterns, the fertilizer mixture integrated with the $CaCO_3$ nanocarrier was confirmed. The particle size, morphology, pore structure, and thermal stability of the $CaCO_3$ nanofertilizer determined through DLS, SEM, TEM, BET, and TGA studies ensures that the material phenomenon of the nanocarrier system is well suited for the progressive uptake of nutrients by plants. In this study, the $CaCO_3$ nanofertilizer is tuned for superior crop cultivation through the higher adsorption of moisture and nutrients necessary for plant growth. The $CaCO_3$ nanofertilizer showed increased rate of observations with groundwater and plant cultivating pot mixture for its physiochemical behaviors, such as fertilizer release, swell rate, equilibrium water content, and water adsorption capacity. The foliar treatment of the $CaCO_3$ nanofertilizer on tomato crops results in the enhanced development of plant growth and yield as observed with plant's fresh/ dry weight and fruit yield of the tomatoes when compared to the $CaCO_3$ nanoparticles, azadirachtin emulsion and panchakavya extract. The

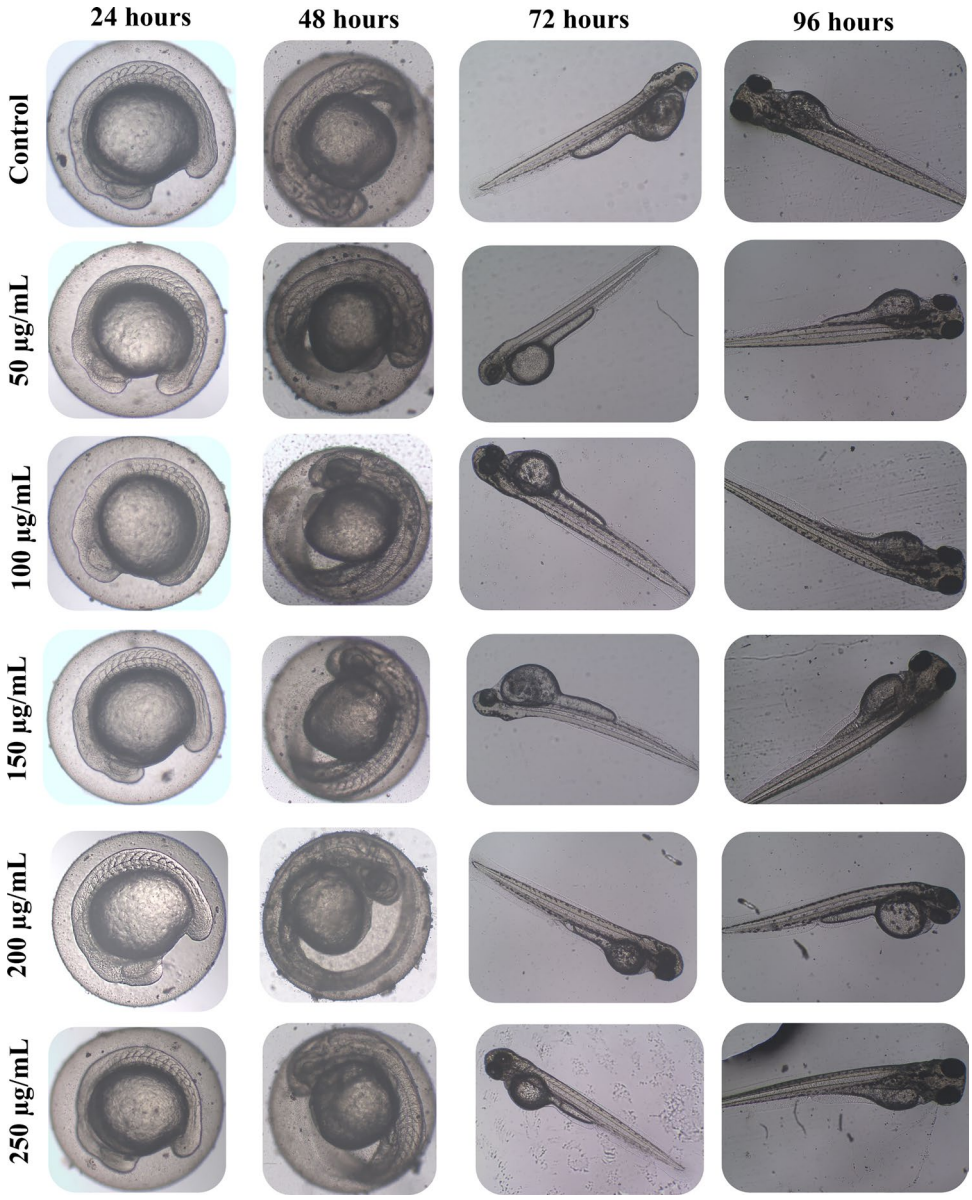


Figure 11. Observation of zebrafish embryo development with exposure to the $CaCO_3$ nanofertilizer in a time and dose dependent manner.

fabricated $CaCO_3$ nanofertilizer exhibits very good antifungal potential against *FOL* fungal organism, which is responsible for wilt disorders in the tomato crops. Further, the tomato extract of nanofertilizer treated groups showed enhanced free radical scavenging activity indicating its antioxidant potential when compared to the individual compounds. The ICP-OES elemental analysis reveals that the foliar treatment superiorly involves increased nutritional uptake and biomass production in tomato crops exposed to $CaCO_3$ nanofertilizer. The zebra fish toxicity studies confirm the biosafety level of the $CaCO_3$ nanofertilizer to the living environmental system In the future, it is hoped to account for the complete microbial entity found in the plant culture medium about the treatment of nanofertilizer, and field comparisons between nanofertilizer and commercial fertilizers can be made. Overall, the results conclude that $CaCO_3$ nanofertilizer formulated will act as a suitable nanofertilizer for smart and sustained agriculture of tomatoes and also for other kind of tropical vegetables by protecting the crops from fungal infections, pest attack and nutritional deficiency.

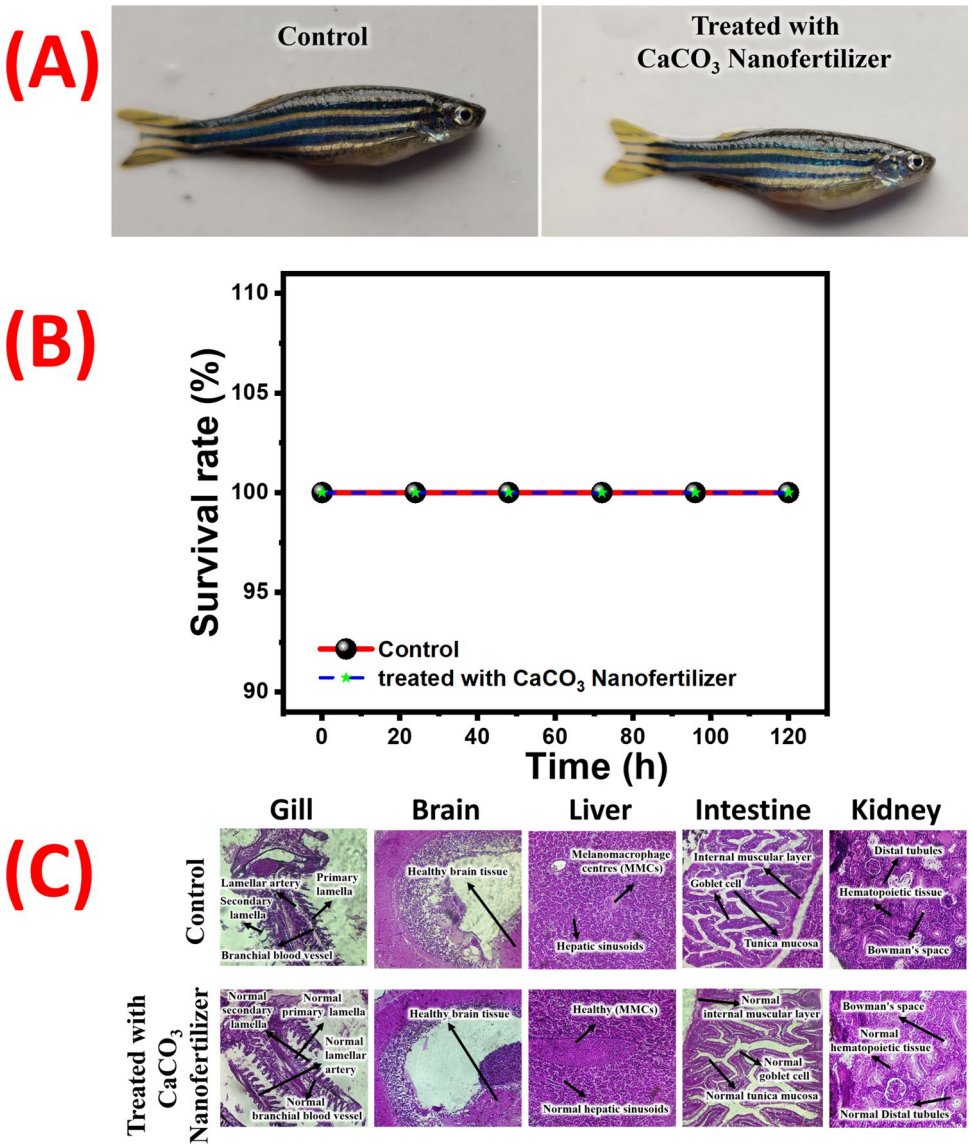


Figure 12. Toxicity evaluations shows the (**A**) physical observation, (**B**) survival rate percentage and (**C**) histopathology images of the tissues of fish gills, brain, liver, intestine and kidney stained with Hematoxylin and eosin for the zebrafish control and treated with $CaCO_3$ nanofertilizer.

Data availability

The corresponding author can provide the datasets presented in this study upon reasonable request.

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References

1. World Health Organization. *A Fact Sheet About Healthy Diet*, April 29, 2020. https://www.who.int/news-room/fact-sheets/detail/healthy-diet
2. United Nations Children's Fund (UNICEF). October 2019. *The State of the World's Children 2019 Children, Food and Nutrition*. (n.d.). https://www.unicef.org/sites/default/files/2019-10/SOWC-2019-EAP.pdf
3. Bokelmann, W., Huyskens-Keil, S., Ferenczi, Z. & Stöber, S. The role of indigenous vegetables to improve food and nutrition security: Experiences from the project HORTINLEA in Kenya (2014–2018). *Front. Sustain. Food Syst.* **6**, 66 (2022).
4. Purcell, A. H. Plant diseases and insects. *Encycl. Insects* **66**, 802–806 (2009).
5. Ofuya, T. I., Okunlola, A. I. & Mbata, G. N. A review of insect pest management in vegetable crop production in Nigeria. *Insects* **14**(2), 111 (2023).
6. Balali, G. I., Yar, D. D., Afua Dela, V. G. & Adjei-Kusi, P. Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. *Int. J. Microbiol.* **2020**, 3029295 (2020).
7. Chatterjee, C. & Dube, B. K. Nutrient deficiency disorders in vegetables and their management. In *Fruit and Vegetable Diseases. Disease Management of Fruits and Vegetables* Vol. 1 (ed. Mukerji, K. G.) 145–188 (Springer, 2004).

8. Aktar, M. W., Sengupta, D. & Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.* **2**(1), 1–12 (2009).
9. Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R. & Wang, M.-Q. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics* **9**(3), 42 (2021).
10. Shaji, H., Chandran, V. & Mathew, L. Organic fertilizers as a route to controlled release of nutrients. *Control. Release Fertil. Sustain. Agric.* **66**, 231–245 (2021).
11. Ye, S., Peng, B. & Liu, T. Effects of organic fertilizers on growth characteristics and fruit quality in Pear-jujube in the Loess Plateau. *Sci. Rep.* **12**, 13372 (2022).
12. Khan, M. S., Akther, T. & Hemalatha, S. Impact of Panchagavya on *Oryza sativa* L. grown under saline stress. *J. Plant Growth Regul.* **36**, 702–713 (2017).
13. Chaudhary, S. *et al.* Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Front. Plant Sci.* **8**, 610 (2017).
14. Kilani-Morakchi, S., Morakchi-Goudjil, H. & Sifi, K. Azadirachtin-based insecticide: Overview, risk assessments, and future directions. *Front. Agron.* **3**, 66 (2021).
15. Ali, M. N., Ghatak, S. S. & Ragul, T. Biochemical analysis of Panchagavya and Sanjibani and their effect in crop yield and soil health. *J. Crop Weed* **7**, 84–86 (2011).
16. Maia, J. T. L. S., Martinez, H. E. P., Clemente, J. M., Ventrella, M. C. & Milagres, Cd. C. Growth, nutrient concentration, nutrient accumulation and visual symptoms of nutrient deficiencies in cherry tomato plants. *Semina Ciências Agrárias* **40**(2), 585 (2019).
17. Silva, G. A. *et al.* Biological performance and oviposition preference of tomato pinworm *Tuta absoluta* when offered a range of Solanaceous host plants. *Sci. Rep.* **11**, 1153 (2021).
18. Zhou, X. *et al.* Cross-kingdom synthetic microbiota supports tomato suppression of Fusarium wilt disease. *Nat. Commun.* **13**, 7890 (2022).
19. Prakashkumar, N., BeemaShafreen, R. & B. S., Jeyakanthan, J., Brindhadevi, K., and Suganthy, N.,. Regenerative marine waste towards $CaCO_3$ nanoformulation for Alzheimer's therapy. *Environ. Res.* **225**, 115631 (2023).
20. Prakashkumar, N., Sivamaruthi, B. S., Chaiyasut, C. & Suganthy, N. Decoding the neuroprotective potential of methyl gallate-loaded starch nanoparticles against beta amyloid-induced oxidative stress-mediated apoptosis: An in vitro study. *Pharmaceutics* **13**(3), 299 (2021).
21. He, X. *et al.* Characteristics and performance of novel water-absorbent slow release nitrogen fertilizers. *Agric. Sci. China* **6**(3), 338–346 (2007).
22. Nadiah Abdul Hamid, N. *et al.* The effect of chitosan content to physical and degradation properties of biodegradable urea fertilizer. *J. Sci. Innov. Res.* **2**(5), 893–902 (2013).
23. Ding, H. *et al.* Nutrients release from a novel gel-based slow/controlled release fertilizer. *Appl. Environ. Soil Sci.* **2016**, 1–13 (2016).
24. Vijay, K. *et al.* Computational biology approaches revealing novel target in vascular wilt pathogen *Fusarium oxysporum* f. sp. Lycopersici for the ligands of marine actinobacterial origin. *J. Pure Appl. Microbiol.* **14**(1), 363–373 (2020).
25. Karny, A. *et al.* Therapeutic nanoparticles penetrate leaves and deliver nutrients to agricultural crops. *Sci. Rep.* **8**, 7589 (2018).
26. Barita, Y., Prihastanti, E., Haryanti, S., Subagio, A. & Ngadiwiyana, A. The influence of granting npk fertilizer and nanosilic fertilizers on the growth of Ganyong plant (*Canna edulis* Ker.). *J. Phys. Conf. Ser.* **1025**, 2054 (2018).
27. Shahzad, T., Ahmad, I., Choudhry, S., Saeed, M. K. & Khan, M. N. DPPH free radical scavenging activity of tomato, cherry tomato and watermelon: Lycopene extraction, purification and quantification. *Int. J. Pharm. Pharm. Sci.* **6**(Suppl 2), 223–228 (2014).
28. Kumari, D., Reddy, M. S. & Upadhyay, R. C. Antioxidant activity of three species of wild mushroom genus Cantharellus collected from North-Western Himalaya, India. *Int. J. Agric. Biol.* **13**(3), 415–418 (2011).
29. Akter, M. K. *et al.* Isolation and quantification of lycopene and determination of B-carotene and total phenolic contents from tomato (*Lycopersicum esculentum*) by using various methods. *Int. J. Food Sci. Nutr. Diet.* **66**, 442–447 (2020).
30. OECD. *Test No. 236: Fish Embryo Acute Toxicity (FET) Test, OECD Guidelines forthe Testing of Chemicals, Section 2* (OECD Publishing, 2013).
31. Song, Y. S. *et al.* Validation, optimization, and application of the zebrafish developmental toxicity assay for pharmaceuticals under the ICH S5(R3) guideline. *Front. Cell Dev. Biol.* **9**, 721130 (2021).
32. Rizzo, L. Y. *et al.* In vivo nanotoxicity testing using the zebrafish embryo assay. *J. Mater. Chem. B* **1**(32), 3918 (2013).
33. Achenbach, J. C., Leggiadro, C., Sperker, S. A., Woodland, C. & Ellis, L. D. Comparison of the zebrafish embryo toxicity assay and the general and behavioral embryo toxicity assay as new approach methods for chemical screening. *Toxics* **8**(4), 126 (2020).
34. Jirankalgikar, N. M., Nariya, P. B., Athavale, A. V. & De, S. Trividha snehapaka of Panchagavya Ghrita: A critical comparative evaluation. *J. Ayurveda Integr. Med.* **4**(2), 107–113 (2013).
35. Caboni, P., Cabras, M., Angioni, A., Russo, M. & Cabras, P. Persistence of azadirachtin residues on olives after field treatment. *J. Agric. Food Chem.* **50**(12), 3491–3494 (2002).
36. Sharma, D. *et al.* Development and validation of stability indicating UV–visible spectrophotometric method for simultaneous estimation of neem (Azadirachtin) and curcumin in pharmaceutical tablet dosage form. *Anal. Chem. Lett.* **9**(4), 564–581 (2019).
37. Chavan, S., Tayade, S., Gupta, V., Deshmukh, V. & Sardeshmukh, S. Pharmaceutical standardization and physicochemical characterization of traditional ayurvedic marine drug: Incinerated conch shell (Shankha Bhasma). *Marine Drugs* **16**(11), 66 (2018).
38. Arumugam, D. G., Sivaji, S., Dhandapani, K. V., Nookala, S. & Ranganathan, B. Panchagavya mediated copper nanoparticles synthesis, characterization and evaluating cytotoxicity in brine shrimp. *Biocatal. Agric. Biotechnol.* **19**, 66 (2019).
39. Zambri, N. D. S., Taib, N. I., Abdul Latif, F. & Mohamed, Z. Utilization of neem leaf extract on biosynthesis of iron oxide nanoparticles. *Molecules* **24**(20), 3803 (2019).
40. Das, P., Sharma, N., Puzari, A., Kakati, D. K. & Devi, N. Synthesis and characterization of neem (*Azadirachta indica*) seed oil-based alkyd resins for efficient anticorrosive coating application. *Polym. Bull.* **78**, 457–479 (2021).
41. Alhammad, B. A. & Seleiman, M. F. Improving plant growth, seed yield, and quality of Faba bean by integration of bio-fertilizers with biogas digestate. *Agronomy* **13**(3), 744 (2023).
42. Pham, T. T. *et al.* Investigation of chitosan nanoparticles loaded with protocatechuic acid (PCA) for the resistance of pyricularia-oryzae fungus against rice blast. *Polymers* **11**(1), 177 (2019).
43. Khan, H. A. *et al.* A performance evaluation study of nano-biochar as a potential slow-release nano-fertilizer from wheat straw residue for sustainable agriculture. *Chemosphere* **285**, 131382 (2021).
44. Sutrisno, L. & Ariga, K. Pore-engineered nanoarchitectonics for cancer therapy. *NPG Asia Mater.* **15**(21), 1–27 (2023).
45. Hammadi, N. I. *et al.* Formulation of a sustained release docetaxel loaded cockle shell-derived calcium carbonate nanoparticles against breast cancer. *Pharm. Res.* **34**(6), 1193–1203 (2017).
46. Shaaban, A., Se, S. M., Mitan, N. M. M. & Dimin, M. F. Characterization of biochar derived from rubber wood sawdust through slow pyrolysis on surface porosities and functional groups. *Procedia Eng.* **68**, 365–371 (2013).
47. Naseem, F. *et al.* Mesoporous $ZnA_{l2}Si_{10}O_{24}$ nanofertilizers enable high yield of *Oryza sativa L. Sci. Rep.* **10**, 10841 (2020).
48. Rivero, A. G., Keutgen, A. J. & Pawelzik, E. Antioxidant properties of tomato fruit (*Lycopersicon esculentum* Mill.) as affected by cultivar and processing method. *Horticulturae* **8**(6), 547 (2022).
49. Shamim, S., Sushmita, N., Darshan, G. & Jidnyasa, R. Effect of post-harvest management on carotenoid content in *Solanum lycopersicum* L., *Carica papaya* L., and *Spinacia oleracea* L. *IJRAR Int. J. Res. Anal. Rev.* **10**(2), 52–68 (2023).
50. Wang, Q., Yang, S., Wan, S. & Li, X. The significance of calcium in photosynthesis. *Int. J. Mol. Sci.* **20**(6), 1353 (2019).

51. Weng, X. *et al.* Calcium regulates growth and nutrient absorption in poplar seedlings. *Front. Plant Sci.* **13**, 887098 (2022).
52. Alzohairy, M. A. Therapeutics role of *Azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. *Evid. Based Complement. Altern. Med.* **2016**, 7382506 (2016).
53. Bajaj, K. K., Chavhan, V., Raut, N. A. & Gurav, S. Panchgavya: A precious gift to humankind. *J. Ayurveda Integr. Med.* **13**(2), 100525 (2022).
54. Kalwani, M., Chakdar, H., Srivastava, A., Pabbi, S. & Shukla, P. Effects of nanofertilizers on soil and plant-associated microbial communities: Emerging trends and perspectives. *Chemosphere* **287**, 132107 (2022).
55. Howe, K. *et al.* The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496**, 498–503 (2013).

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Author contributions

P.N.: Conceptualization, Formal analysis, Data curation, Validation, Methodology, and Writing – original draft.
S.N.: Supervision, Funding acquisition, Project administration, Writing – review and editing and validation.

Competing interests

The authors declare no competing interests.

Additional information

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