PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(1): 1-7
https://doi.org/10.14719/pst.4120

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RESEARCH ARTICLE

Effect of gamma rays induced mutagenesis on the agronomical and biochemical traits of acid lime

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OPEN ACCESS

ARTICLE HISTORY

Received: 16 June 2024
Accepted: 29 September 2024

Available online
Version 1.0 : 05 December 2024
Version 2.0 : 01 January 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Manjusha MR, Rajangam J, Saraswathy S, Venkatesan K, Rajesh S, Madhan Mohan M, Gnanasekaran M. Effect of gamma rays induced mutagenesis on the agronomical and biochemical traits of acid lime . Plant Science Today. 2025; 12(1): 1-7. https://doi.org/10.14719/pst.4120

Abstract

One of the indigenous citrus crops of India is acid lime, which has a high economic value and unique flavour compared to other citrus fruits. The current study was intended to ascertain the gamma ray dosage that proves lethal for the acid lime variety PKM1. The acid lime seeds were exposed to nine distinct gamma radiation dosages ranging from 5 to 45 Gy with an interval of 5 Gy and compared to untreated control. Different irradiation doses demonstrated substantial differences in morphological and biochemical traits. Increased dosage of gamma radiation leads to significant changes in various agronomic parameters of plant germination, number of plants, plant height, and change in biochemical traits. Various gamma radiation doses revealed a low survival rate after 35 Gy, with LD 50 at 27.86 Gy.

Keywords

gamma irradiation; lethal dose; PKM 1; survival and mortality

Introduction

Acid lime (Citrus aurantifolia Swingle) is one of the major fruits of India, successfully cultivated on a wide range of soils owing to its comparative tolerance to abiotic stress and high yield. In India, 2,546 million tonnes of acid lime are produced in an area of 252 million hectares (1), and acid lime cultivation covers 10,000 hectares across Tamil Nadu (2). It is a significant fruit crop that has high nutritional value and an affordable price. Acid lime is rich in nutrients, including calcium, phosphate, iron, vitamin C (62.9 mg/100 mL), as well as vitamins B1 and B2 (3). The usefulness of fruits in Indian cuisine extends beyond their medicinal and nutritional advantages to encompass their culinary and value-added products (4, 5).

Nowadays, acid lime is gaining popularity among citrus farmers because of its wide adaptability to various climatic conditions, minimal investment costs, bearing of fruit habit, keeping quality of fruits, and increasing constant demand in the market (6). However, due to limited land resources, lack of irrigation water, labor, and input prices, it is vital to optimize productivity. These conditions need the use of rigorous horticultural practices to attain the optimum outcomes. Among other factors, improved productivity of acid lime can be achieved through the development of released high-yielding and resilient varieties to biotic and abiotic stress. This can be achieved through the creation of genetic variation in the species. One of the better options is to create genetic variations using chemical mutagens or ionizing radiations, as these compounds generate more chromosomal alter-

ation (7). For several features of commercial importance, there was significant variation in the mutant population. Species variability/variation can create new genotypes with improved productivity.

Previous studies have investigated mutagenesis in citrus species, resulting in the formation of seedlessness (8,9), disease resistance (10), LD50 determination (11), sparse-seeded mutant (12), ornamental fruits (13), low-seeded mutants (14), and creation of novel or new mutants (15). The physical mutation using ionizing radiations (Gamma rays, X-rays, alpha, and beta particulates along with the neutrons and protons) (16) has been widely employed in mutation breeding in fruit crops (17). Plant mutation breeding of acid lime plants could aid in the acquisition of desirable traits as well as enhance economic value. Physical mutagens have several advantages over chemical mutagens, including precise dose determination, which enables sufficient reproducibility and high and homogeneous penetration into plant tissues, especially for gamma rays (18). There are not many studies that are connected to seed irradiation's influence on agronomic and biochemical studies. At the morphological stage, the variants of mutants are observed. The PKM 1 mutants exhibit distinct changes in leaf morphology, reduced thorns, and semi-dwarf stature. The findings offer notable alterations to the horticultural characteristics of plants, which could revolutionise lime cultivation methods and improve agricultural production, efficiency and sustainability also provide significant information for lime growers and agricultural researchers. Hence, this work was carried out.

The study has the following objectives: to find the optimal gamma irradiation dose and the impact of gamma radiation on morphological and biochemical features after irradiation exposure. The outcomes of this research could be applied to large-scale mutagenesis breeding programs for the creation of diverse acid lime mutants.

Materials and Methods

The research was conducted at the Horticulture College and Research Station, Tamil Nadu Agricultural University, Periyakulam, India, to ascertain the appropriate dosage to trigger the acid lime mutation. Ten treatments with varying dosages of gamma rays (5 Gy to 45 Gy, with the interval of 5 Gy), as well as a control, were undertaken at Atomic Research (IGCAR), Kalpakkam, Chennai, using a Co^{60} gamma source. The various dosages were automatically calculated based on the Co^{60} gamma source's half-life.

Seeds from PKM1 fruit were manually extracted and collected in a beaker containing water. Seeds that do not have embryos are discarded and only the sunken seeds are utilised for the research. For each treatment, 100 seeds were collected and treated with gamma rays. Following exposure, the treated seeds were planted in nursery polythene bags alongside untreated seeds as a control with a mixture of red soil, sand, and FYM (2:1:1) and kept under controlled conditions in the nursery. Water was sprayed over the planting area to maintain a regulated environment and to supply the seedlings with enough moisture to germinate.

Agronomical observations

Germination percentage

Four weeks after sowing, the number of germinated seedlings were recorded, and the germination percentage was computed as follows.

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \text{ X } 100$$

……..(Eqn. 1)

Survival percentage

The survival rate is calculated by counting a number of seedlings that remained alive after an eight-week period.

$$\text{Survival percentage (\%)} = \frac{\text{Number of plants that survived after germination}}{\text{Total number of germinated seeds}} \text{ X } 100$$

……..(Eqn. 2)

Plant height (cm)

The height of every plant in each treatment was measured at monthly intervals from the lowest point of the stem towards the tip, and the mean height was specified in centimeters.

Number of leaves

The number of leaves on every plant in each treatment was counted, and the mean number of leaves for each plant was calculated.

Leaf length and width (cm)

The length of the leaf was measured at the full growth stage of fresh leaves and measured in centimeters.

Internodal length (cm)

The internodal length between the nodes of all plants was measured in each treatment, and the mean was reported in centimeters.

Relative water content

According to Barrs and Weatherley (19), leaf discs were removed from the foliage, avoiding the veins and making sure leaf discs were sizable. Five samples per replication were taken as a single treatment, and all samples were pre-weighed (W). Allowed the discs to float in a petri plate filled with distilled water at ambient temperature for 2 hours, then allowed the tissues to air dry slightly and re-weighed (TW). After 24 hours of oven drying at 80°C, the samples were weighed till the weight remained constant (DW) and substituted in the equations.

$$\text{RWC (\%)} = \frac{W-DW}{TW-DW} \times 100$$

…….(Eqn. 3)

Where, W –Fresh weight of the sample, DW – Dry weight of the sample, TW – turgid weight of the sample

Chlorophyll content

In order to measure chlorophyll, one gram of fresh leaves was weighed and blended with an adequate amount of 80% acetone in a mortar and pestle. The supernatant was

decanted and filtered, and 10 mL of 80% acetone was used as the extracting solvent. The homogenized sample was centrifuged at a rate of 10,000 rpm for fifteen minutes at a time. After collecting the supernatant of 0.5ml, added 4.5ml of solvent, and Chlorophyll a and b were measured by spectrophotometer at the absorbance at 663 nm and 645 nm (20).

Chlorophyll 'a' (mg/g) = 12.7(A663) - 2.59 (A645)

Chlorophyll 'b' (mg/g) = 22.9 (A645) – 4.68 (A663)

Total Chlorophyll (mg/g) = 8.2 (A663) + 20.2 (A645)

……..…..(Eqn. 4)

Fresh weight and Dry weight (g)

The fresh weight of leaves was measured using an electronic balance, and mean values were computed and represented in grams. After weighing, the leaves were completely dried in a hot air oven at 60°C until they reach a consistent weight and were expressed in grams.

Proline content (µg/mg)

An amount of 0.5 grams of plant material was homogenised with 3% of 10 ml aqueous sulphosaliyic acid and then filtered it. Placed 2 ml of the filtrate into a test tube, then added 2 ml of ninhydrin and glacial acetic acid each and heated for an hour in a hot bath. The reaction was terminated by placing it in an ice bath, followed by adding 4 ml of toluene and then stirring for 20 to 30 minutes. Red intensity was measured at 520 nm, thereby preparing the standard in the same manner (21).

Proline content per g of the sample = -

$$\frac{\text{Microgram proline /ml} \times \text{1ml toluene}}{115.5} \times \frac{5}{\text{1g sample}}$$

…….. (Eqn. 5)

Leaf soluble protein (mg 100g $^{-1}$)

Acid lime leaf (1g) was first homogenised in 10ml of phosphate buffer, and the supernatant was then collected by centrifugation, and diluted with buffer solution to make 100 ml. Sample extracts of 0.1 ml and 0.2 ml were placed in separate test tubes and diluted with distilled water to make a total of 2 ml. To the extract, added Alkaline copper solution (reagent C) in each test tube of 5 ml and kept for 10 minutes after mixing, followed by adding reagent D (Folin Ciocalteau reagent) 0.5 ml, which remained dark for 30 minutes. The absorbance at 660 nm was measured against a blank, and the protein content in 100 grams of leaves was calculated using the standard graph method (22).

Protein =

(Graph value × Volume of the test × Total volume of the extract) / (Weight of the sample × 100).

……..(Eqn. 6)

Statistical Analysis

Using probit analysis, the lethal dose (LD_{50}) values of gamma irradiation in acid lime variety PKM1 have been determined based on the germination percent. In order to determine the significance of the plant responses observed at various treatments, SAS statistical computing software was used to conduct conventional analysis of variance in the data. The investigation was carried out using a completely randomized design.

Results

The LD_{50} for gamma radiation was determined by assessing seed germination and survival rates after exposure to various doses of gamma rays. The data were analyzed using probit analysis, comparing the results to an untreated control (Table 1). The seed survival rates in PKM1 declined steadily as the gamma dose increased and maximum reduced at higher dosages of 45 Gy. The probit curve analysis in the current study revealed that the LD_{50} value for gamma rays was 27.86 Gy for PKM1 acid lime (Fig 1).

Impact of mutagens on Agronomic characteristics

Gamma radiation effects on the M_1 population of acid lime *var.* PKM 1 are presented in (Fig. 2). In comparison to the control, increasing gamma radiation decreases the germination rate, survival percentage, plant height, and plant count. This is due to high gamma radiation exposures produced by radio inhibition by affecting growth promoters, which can lead to tissue death and regeneration (23). A similar finding was observed in rough lemon. Depending on the intensity of irradiation, a survival plant can repair itself and continue to grow after being exposed to gamma rays (24). The decrease in seed germination at higher

Table 1. Determining LD_{50} value for Acid lime PKM 1 by probit analysis.

Dose (Gy)	Log_{10} of doses	Germination percentage	Percent reduction over control	Observed mortality (%)	Corrected mortality (%)	Empirical probit units	LD_{50} value
0	-	91.00	-	9	00	-	
5	0.70	90.00	1	11.00	2.2	2.99	
10	1.00	83.00	8	17.00	8.8	3.65	
15	1.18	78.00	13	22.00	14.3	3.93	
20	1.30	70.00	21	30.00	23.1	4.26	27.98
25	1.40	55.00	36	45.00	39.6	4.74	
30	1.48	40.00	51	60.00	56.0	5.15	
35	1.54	26.00	65	74.00	71.4	5.57	
40	1.60	18.00	73	82.00	80.2	5.85	
45	1.65	13.00	78	87.00	85.7	6.07	

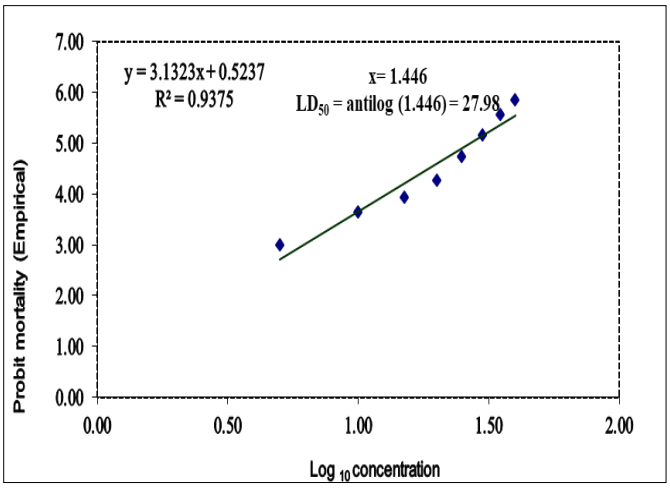

Fig. 1. Probit computation on the basis of corrected mortality rates of Acid lime variety PKM 1

mutagen dosages could be attributable to cellular disturbances. It is possible that mutagenesis treatments produce molecular damage to cell components or altered enzyme performance, which results in diminished seed germination (Fig 3). The reduction in plant height could be attributed to delayed and inhibited germination. It could possibly be due to changes in physio-biochemical pathways connected to gibberellic acid activity, which may limit plant cell activity and reduce mitotic cell division and elongation at higher dosages, killing or destroying meristematic cells (25). Lower doses of irradiation had a positive impact on growth and multiplication; however, greater doses had a deleterious effect on growth, resulting in a higher mortality rate and an overall reduction in growth (26).

Effects of mutagens on biochemical characteristics

The gamma radiation led to changes in the biochemical

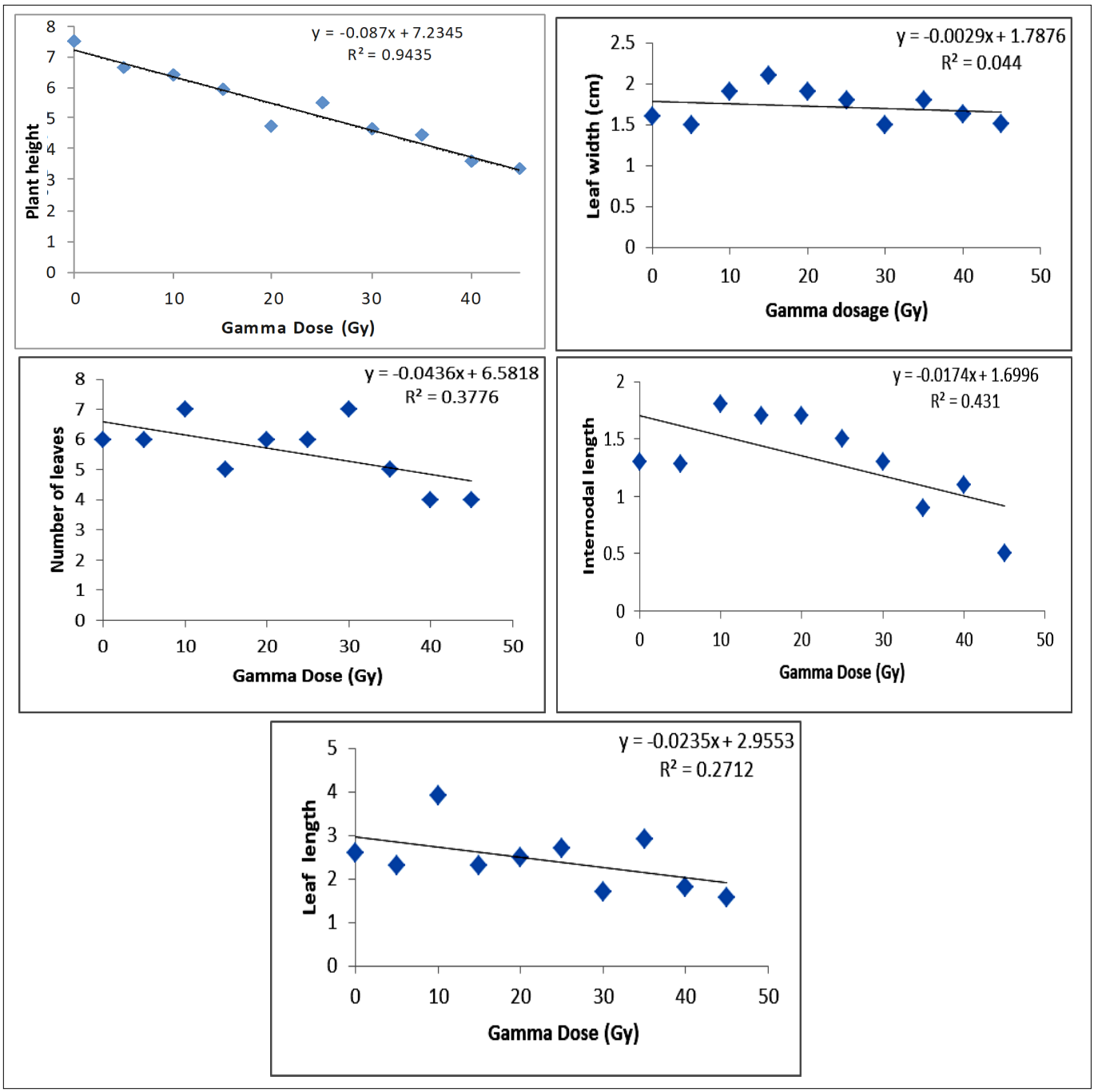

Fig. 2. Influence of gamma radiation on vegetative parameters of Acid lime PKM 1.

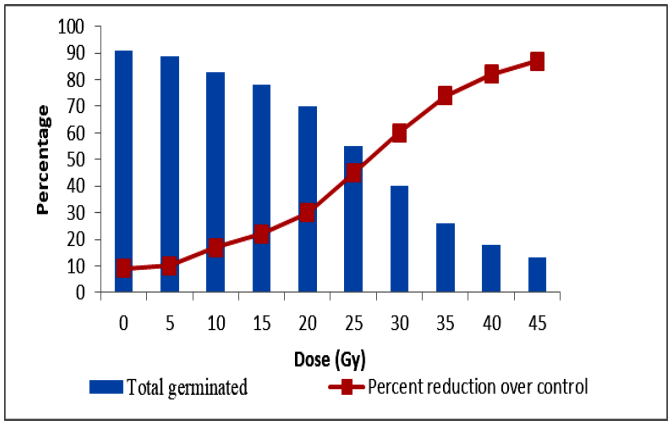

Fig. 3. Effect of gamma radiation in germination of Acid lime variety PKM 1.

parameters of plants, as represented in (Table 2). In mutagenesis-induced mutants resulting from gamma radiation exposure, the relative water content of leaves improved linearly with increasing dose. This may be because the mutants have thicker cuticles and more wax deposited on them, relating to the same findings in Kinnow mandarin (15). A crucial and necessary component of the leaf photosystem is chlorophyll concentration, which is one of the important criteria for measuring a plant's photosynthetic efficiency. In our research, higher radiation dosages were associated with a reduction in chlorophyll concentration.

Table 2. Effect of gamma radiation on biochemical parameters of Acid lime PKM 1.

Dose Gy	Relative water content (%)	Chlorophyll content (mg/100g)	Fresh weight (g) /Dry weight (g) of leaves		Soluble protein (mg/g)	Proline content (µM/g)
			FW (g)	DW(g)		
0	77.00	1.72	0.692	0.270	11.12	0.21
5	73.00	1.68	0.662	0.295	12.41	0.46
10	69.00	1.63	0.749	0.328	13.03	0.50
15	83.00	1.43	0.532	0.230	16.36	0.25
20	89.00	1.66	0.774	0.277	15.74	0.29
25	86.00	1.59	0.769	0.345	16.64	0.21
30	94.00	1.49	0.779	0.264	17.33	0.24
35	86.00	1.41	0.588	0.170	17.60	0.22
40	84.00	1.45	0.563	0.212	15.45	0.24
45	81.00	1.40	0.549	0.208	15.12	0.20
SEd	1.85	0.03	0.015	0.006	0.309	0.006
CD@5%	3.87	0.06	0.03	0.01	0.64	0.01

This could be because a greater mutagenesis dose causes a reduction in photosynthetic pigments because of pigment degradation and chloroplast damage, which would lower catabolic and enzyme activity, as studied in papaya (27) and grapes (28,29). The total soluble protein content of the mutants showed that the higher dosage mutants accumulated more protein in their leaves, but the mutants treated with lesser doses showed a discernible decrease in leaf protein content. This might be caused by alterations to protein synthesis brought on by the synthesis of increased amino acids at lower dosages, and conversely, at higher doses, these findings matched those of pomegranate (30) and *Citrus sinensis* (31). In the current investigation, a substantial rise in proline content was noted as the radiation dose increased, which highlights the significance of proline as a compatible solute in stressful situations, as observed in bananas (32) and pomegranates (30). Higher gamma ray concentrations caused the fresh weight of leaves to increase. This is probably because plants' water content depends on the availability of water in their environment, as similarly, reported in acid lime (33).

Conclusion

The duration of gamma irradiations and the intensity of the radiation determine the morphological and biochemical changes in plants. The inhibitory impact may be brought on by the fact that greater dosages of gamma irradiation produce biological damage at a faster rate, while the lower concentrations increase enzyme and growth hormones, which are responsible for quality characteristics. The optimum dosage is 25-30 Gy, while higher gamma radiation doses of 35 to 45 Gy had an impact on acid lime growth, survival, and 78% germination reduction over control. Mutation has been proven to generate genetic variation in acid lime and speed up the breeding program. The selection of PKM 1 mutants with variations has resulted in a range of distinctive morphological features, including a reduced height, altered leaf form, and fewer thorns. This genetic gain and the variability of commercially valuable mutants should be stabilised by forwarding it to the next generations.

Acknowledgements

The authors express sincere gratitude to the Advisory Committee, Horticulture College and Research Institute, Periyakulam (Tamil Nadu) India and Indira Gandhi Centre for Atomic Research (IGCAR), Radiological and Environmental Safety Division, Kalpakkam for their valuable support and assistance throughout the research work.

Authors' contributions

MR carried out the research work, statistical analysis and drafted the manuscript, JR participated in Conceptualization and methodology, SS did validation and resources, KV analysed results of experiments, reviewed and edited, SR Drafted, reviewed and edited, MM Investigated and conducted the critical revision, MG review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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