Received: 1 October 2020 | Revised: 8 January 2021 | Accepted: 18 January 2021

DOI: 10.1049/nbt2.12027

IET Nanobiotechnology

The Institution of Engineering and Technology WILEY

ORIGINAL RESEARCH PAPER

Statistical approach to synthesise biogenic silica nanoparticles from rice husk and conjugated with *Justicia adhatoda* extract as green, slow-release biocide

Peerzada Gh Jeelani | **C Ramalingam**

Department of Biotechnology, School of Biosciences and Technology, Nano-Food Research Group, Instrumental and Food Analysis Laboratory, Division of Industrial Biotechnology, Vellore Institute of Technology (VIT), Vellore, Tamil Nadu, India

Correspondence

Ramalingam Chidambaram, 115 Hexagon building Industrial Biotechnology Division, School of Bioscience & Technology, Vellore Institute of Technology (VIT), Vellore 632014, Tamil Nadu, India.

Email: cramalingam@vit.ac.in

Abstract

Biogenic silica synthesised from rice husk was used as a controlled release system of an eco-friendly biocide consisting of a *Justicia adhatoda* extract. Fourier-transform infrared spectroscopy (FTIR) indicated the presence of ester bonds between the silica support and the conjugated *Justicia adhatoda* extract. Surface area analysis and microscopy confirmed a high level of *Justicia adhatoda* extract loading in the silica support. The phytochemical investigation of *Justicia adhatoda* was done by Gas chromatography–mass spectrometry (GC-MS) spectroscopy. Moreover, compared with the naked biogenic silica nanoparticles, a better thermal stability was determined for the conjugated system of the extracted compounds. Trial of kinetic release of silica: *Justicia adhatoda* ~29% of loaded *Justicia adhatoda* was released within 1 h and then the rate of release became slow. Net release of *Justicia adhatoda* was observed up to 50% within 7 h. The *Justicia adhatoda* compounds released from silica also showed the improved mortality rate against stored product pest rice weevil (*Sitophilus oryzae*).

1 | INTRODUCTION

Wide range of biocides such as the azoles, carbamates, pyrethroids, cupric, aromatics and halogenated heterocycle compounds have been widely applied to decrease crop losses due to pests, and to improve the service life of stored grains. *Justicia adhatoda L.* (*bashok*) is a tall dense evergreen shrub belonging to the family *Acanthaceae*. Many scientific studies have indicated that *J. adhatoda* has antibacterial, antifungal, anti-asthmatic, antihistaminic, anti-inflammatory, anti-ulcer, antioxidative, antitubercular, antitussive, larvicidal, anti-Alzheimer and hepatoprotective effects [1]. Extracts from *J. adhatoda L.* (*Acanthaceae*) strongly reduced the fitness of the mosquito, Aedes aegypti Linn. The methanolic extracts inhibited several enzymes responsible for protecting insects from oxidative and other damages, including glutathione-S-transferase, superoxide dismutase, cytochrome P450 and α- and β-esterases. They increased repellency (maximum repellency at 100 ppm) in host-seeking adult females regardless of high efficacy of these organic biocides (*Justicia adhatoda*) in containing pests and mites [2]. But, there is a concern related to their bioavailability and stability in different temperature regions [3].

The rice weevil *Sitophilus oryzae L.* (Colepotera: Curculionidae) is a dominant pest of various food grains like rice, wheat and maize under storage [4]. Synthetic insecticides have been found effective against stored product pests but proved to be hazardous to environment as well as to humans and domestic animals. The over dependence and over usage of synthetic pesticides especially insecticides since last four decades led to wide spectrum of pests problem like pests resistance to chemicals, resurgence of pests, residues in food and soil and risks to human and animal health, besides environmental pollution [4]. The presence of unusually large numbers of insects in the stored grains remains the foremost challenge all over the world. No scrupulous nontoxic insecticide for the fortification of these insects exists in the market. Unremitting exposure to the toxic chemicals that are used as insecticides may cause several disorders. There are about 20,000 deaths in the third world countries annually according to World Health Organization (WHO) due to pesticide poisoning. Chemical fumigants and residual insecticides are predominantly used for

fortification of stored grains against pest infestation [5]. Grievously, this spearheads to adulteration of food with toxic pesticide residues. The expansion of resistance in both field and stored insects to traditional insecticides coupled with greater consumer alertness of the repercussion of their residual toxicity, and environmental contamination has to steer agro-chemical researchers to appraise the use of inert dust as a surrogate insecticide for crop fortification. Besides, voluminous stored-product insects have become resistant to a range of chemical pesticides [6]. *Sitophilus oryzae* is a prevalent insect pest ravaging stored grains worldwide. *Sitophilus oryzae* vernacularly called as rice weevil is the utmost conventional pest found in stored rice, wheat, corn, buckwheat and oat. The nuisance of this pest leads to colossal damage in the quality as well as quantity of stored grains. Conventionally fumigants (methyl bromide and phosphine) and chemical treatment (organo-phosphates) are used to control this pest. However, this leads to the accumulation of the toxic residue of pesticide in the whole grains [7, 8]. Rice weevil (*S. oryzae)* fosters larvae inside the kernel and, consequently, is shielded from grain protectant residues on the peripheral of the kernel. *S. oryzae* is becoming resistant to phosphine and conventional insecticides such as pyrethroids [9–11]. The exploitation of nanoparticles as the pesticide is an unconventional approach to combat pests, which have become resistant to conventional pesticides. It is catalogued as a primary pest which insinuates that insects are competent of infesting whole grain kernels. Consumer perception of the global warming and consequences of residual toxicity and escalating resistance of insects to storage insecticides has preceded the researchers to evaluate alternative approaches for the waste management of crop residues and to protect stored grains [12]. Also, improved biocide efficiency is needed to increase the bioavailability and stability of bioactive compounds of the crude extract for enhanced life performance and avert excessive initial dosages or frequent applications.

There have been several attempts to control the release rate of biocides by encapsulating them mainly in polymeric carriers. Biodegradable and synthetic polymers can be used as release rate controlling carrier materials of biocides; however, the polymeric carriers are expensive due their synthesis and precursors, and they are thermally, dimensionally and chemically unstable [13, 14]. Biogenic silica nanoparticles could be used as an attempt to design cheaper and cleaner controlled release systems compared to those prepared with polymeric and synthetic silica as carrier material. Economical and renewable silica nanostructures can be obtained from biorefinery platforms of silica-accumulating plants, Rice husk is rich in silica content accounts during its combustion, about 20–25 wt% of rice husk ash (RHA), containing more than 90% silica [15, 16]. The Sol–gel method is a common route in the synthesis of silica nanoparticles due to its ability to form high purity and homogenous products at proper conditions. Tetraethyl orthosilicate (TEOS) is generally used as a precursor in alkaline medium to produce silica nanoparticles. Sodium silicate is another alternative precursor for synthesis of silica nanoparticles. Sodium silicate bids characteristic advantages over TEOS including refined and uniform particle size along with the high concentration of silica nanoparticles. Many agricultural wastes have been used to extract sodium silicate such as sugarcane bagasse ash (SBA), rice husk , groundnut shell, banana peel, coconut husk, orange peel and walnut shell as an economical and non- metallic bio-precursor for biogenic nano-silica synthesis [17, 18].

Silica nanoparticles have also been immensely explored due to their interesting properties such as hydrophilic surface favouring protracted circulation, versatile silane chemistry for surface functionalisation, excellent biocompatibility, ease of large scale synthesis and porosity [19].

Gangwar et al. reported the conjugation of curcumin with silica nanoparticles for controlled kinetic release which increases the bioavailability of the curcumin molecule by also keeping its therapeutic activity intact [20]. Manju et al. have also reported synthesis of water-soluble gold nanoparticles in curcumin−polymer conjugate and studied it for blood compatibility and targeted drug delivery onto cancer cells [21]. Mattos et al. reported a green and non-bio-accumulating slow release biocide system consists of silica nanoparticles loaded with neem extract, which showed comprising results neem compounds released from silica showed a satisfactory scavenging activity over 50% of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical, and could cause the mortality of the majority of workers of *Acromyrmex crassispinus*, an ant species .

The optimisation of biogenic silica nanoparticles synthesis luxuriating response surface methodology (RSM) has been testified in the surfactant-free synthesis of high surface area silica nanoparticles derived from rice husks [22]. This approach has advantages of shaping the ideal reaction conditions illustrating the fewer experimental results than those needing prerequisite to feedback the consequence of inclusive input variables. The central composite design (CCD) method was used to regulate the number of experiments to be evaluated for the optimisation of the variables and responses. The minimum, intermediate and maximum values of each variable were labelled as −1, 0 and +1, respectively. In this paper, the authors report a neat protocol which outlines the conjugation of *Justicia adhatoda* with biogenic silica nanoparticles by using a simple wet chemical method. This protocol foresees good bioavailability of the *Justicia adhatoda* extract. Also, the study shows potential application of such a conjugate in the agricultural domain.

2 | MATERIALS AND METHODS

Justicia adhatoda extract was used as an entomotoxic agent. The extraction was done by the traditional method of Soxhlet extraction with hexane solvent (high media). The phytochemical investigation was performed by Gas chromatography–mass spectrometry (GC-MS) analyses of hexane extract of *Justicia adhatoda* using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 600, USA) equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 mm i.d, film thickness 0.25 μm) (Figure 1). Biogenic nano-silica were synthesised following a previously described extraction procedures [22, 23]. Rice husk was collected from the farms of Lalapet village, Vellore District. Rice husk was cleaned thoroughly to

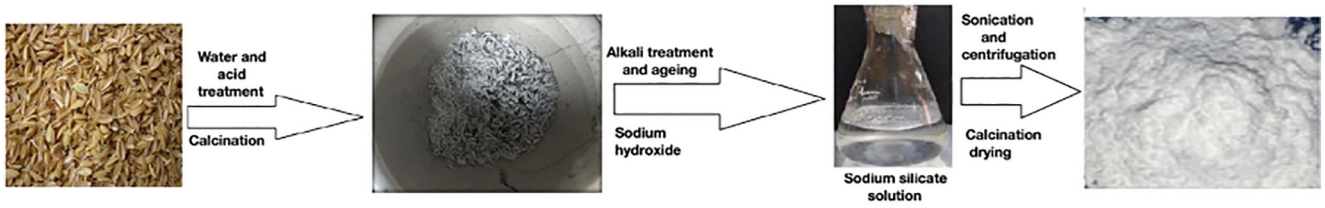

FIGURE 1 Biogenic synthesis of nano silica from rice husk

remove all remaining dirt and dust by boiling in the water on a hot plate with magnetic stirrer several times, then dried in a hot air oven. Dried rice husk was pre-treated with different acids (1 N HCl, 1N HNO_3, 1 N H_2SO_4) and kept in the water bath at 75°C for 30 min to remove ionic and other impurities further washed with distilled water 2–3 times and further kept in a hot air oven again for complete drying. The treated rice husk was incinerated in the muffle furnace at 600°C under atmospheric conditions for 4 h. For extraction of the silica, 1 g of prepared powdered ash was stirred with 100 ml of 1 N NaOH solution for 1 h at 60°C and filtered. The silica present in the ash combines with the sodium present in the NaOH solution and forms sodium silicate (Na_2SiO_3) solution. At room temperature, the pH of sodium silicate (Na_2SiO_3) filtrate was adjusted to pH 7.4 using 20% H_2SO_4. The reaction of sodium silicate with sulphuric acid forms a white silica precipitate solution.

Formation of sodium silicate solution from ash

$$SiO_2+2NaOH = Na_2SiO_3+H_2O$$

The solution was kept under continuous stirring for 48 h. The stirred precipitate solution was sonicated for 10 min and centrifuged at 10,000 rpm for 20 min and washed multiple times with ethanol to remove all the adulterations. The precipitate was then dried in a hot air oven at 80°C overnight. After complete drying, white powdered nano-silica was formed. It was ground with mortar and pestle to get a fine powder. Sintering process was carried out by heating the synthesised nano-silica in an electric furnace at 900°C under atmospheric conditions for 2 h (Figure 1).

Formation of silica dioxide from sodium silicate solution

$$Na_2SiO_2+H_2SO_4=SiO_2+Na_2SO_4+H_2O$$

Procedure for the synthesis of silica: *Justicia adhatoda* conjugate, 50 mg of plant extract was added to sodium silicate (Na_2SiO_3) solution formed and continuously stirred, heated at 60°C for next 60 min. The final colour of the solution was dark green. After this, the reaction flask was placed in an oven at 40°C for 24 h to evaporate the solvent. Powder was collected and washed three times in deionised (DI) water. The colour of the powder was found to be green colour.

Optimisation of biogenic silica nanoparticles synthesis using response surface methodology was used to study the effect of overall input variables and to obtain maximum production. The RSM using CCD was applied to augment the several factors namely rice husk concentration, calcination time and calcination temperature for enhancing the synthesis of silica nano-powder. The quadratic model was utilised to probe the data. Each factor in the design was premeditated at five different levels (−1, +alpha, 0, +1, −alpha) and the minimum and maximum ranges of rice husk concentration, calcination time and calcination temperature variables were established as shown in Table 1.

A design with 20 experiments was framed, and trials were carried out in 200 ml alumina crucibles (high media) at maximum temperature of 1000–1500°C with different parameters of rice husk concentration, calcination time and calcination temperature. Synthesised biogenic nano-silica was used as a response. The 3D interactions and contour plots were computed to appraise the augmented parameters, which influence the response. By using a second-order polynomial equation, all the responses were analysed, and the data were fitted to the equation by multiple regression procedures. To validate the predicted value and the experimental value of the responses, an experiment was conducted in triplicates using the optimum values for variables given by response surface optimization. The experimental design results were analysed and interpreted using Design-Expert version 7.0 statistical software (Stat-Ease Inc.).

Thermogravimetric experiments were performed in TG/DSC, with Ar atmosphere using 20 mL min^{-1} gas flow. Morphology of the silica nanoparticles, biocide system and extract. The surface morphology and elemental composition of the biocide system was measured by transmission electron microscope (Transmission Electron Microscope (TEM)–FEI-Tecnai G2 20 Twin) with an accelerating voltage of 200 kV and scanning electron microscope (SEM) with EDAX (SEM-EDAX) device EVO 18. The functional groups of the, silica nanoparticles, biocide system and extract were attained by utilising FTIR spectroscopy over the wavenumber range from 4000 to 400 cm^{-1}. In vitro release kinetics of *Justicia adhatoda* from silica nanoparticles was studied using UV−vis spectroscopy and is shown in Figure 7. For this purpose, silica: *Justicia adhatoda* conjugate was dispersed (2 mg/ml) in the phosphate buffer solution (PBS: 7.4 pH) and an experiment was performed at 35°C. The Malvern Mastersizer particle analyser was

TABLE 1 Independent factors and its level used in response surface central composite design for synthesis of silica nanoparticles (response)

Factor	Name	Units	Low actual	High actual	Low coded	High coded	Mean	SD
A	Concentration of Rice husk	g	50.00	90.00	−1.000	1.000	70.00	16.527
B	Time	h	3.00	6.00	−1.000	1.000	4.500	1.240
C	Calcination temperature	°C	400	700.00	−1.000	1.000	550.000	123.951

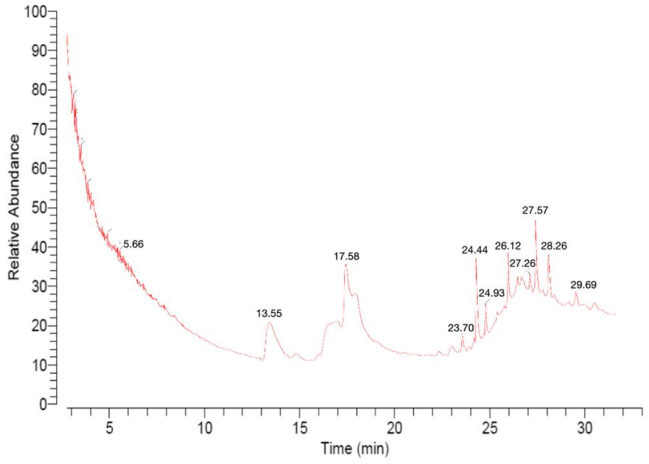

FIGURE 2 Gas chromatography–mass spectrometry (GC-MS) profile of hexane leaf extract of *Justicia adhatoda*

used to analyse the particle surface dimensions of the biogenic synthesised nano-silica and silica: *Justicia adhatoda* conjugate.

The entomotoxicity bioassay on *S. oryzae* was conducted in multipurpose clinical sample collectors, which are small plastic screw-capped jars purchased from Himedia. The caps were pierced to permit aeration. Each jar was filled with 5 g of rice (IR64). Insecticidal effects on treated grain. Bioassay was conducted by applying 1 mg of each sample (silica nanoparticles, conjugated silica nanoparticles, *Justicia adhatoda* extract onto 5 g of rice grains). The control was only treated with 1 ml of acetone. The treated grains were dried under the fume hood. Twenty Weight unsexed adults of *S. oryzae* are introduced into the respective petri dishes containing 5 g of treated rice. All treatments were monitored to obtain the mean percentage of mortality after 72 h. Experiments were replicated for four times.

The tested insects were respectively subjected to acetylcholinesterase biochemical assay (AChE). Each insect was removed and homogenised at 0°C in 0.05 M-phosphate buffer, pH 7.5 (KH_2PO_4-NaOH) (Sigma) using a glass homogeniser pestle (5%, w/v, homogenate). Plastic tube covered with ice was used to keep the homogenised mixture. The homogenates were centrifuged at 16,000 rpm for 20 min (5°C). These supernatants were kept in the ice and used without further purification for studying the acetylcholinesterase and its inhibition. To determine the AChE inhibition activity, procedure from Arshia Hematpoor et al., 2017 [24] is used. 10 µl of enzyme supernatants were transferred to each well of a 96-well microtiter plate using electronic multichannel pipette (High Media), then mixed with 20 µl of tested compounds in solution form and 150 µl of phosphate buffer which were kept at 1–4°C temperature were added. Biogenic nano-silica, silica: *Justicia adhatoda* conjugate and *Justicia adhatoda* plant extract were dissolved in DMSO (Sigma) as AChE inhibitor and 0.1% DMSO (v/v) was added into test well in separated rows. The microtiter plates were incubated for 10 min at 25°C. Then, 20 µl of acetylthiocholine iodide (ACTHI) (Sigma-Aldrich) (0.4 mM) and DTNB (Sigma-Aldrich) (0.3 mM) were added to enzyme supernatants solution to observe the reaction. During the 30 min reaction at room temperature, yellowish or colourless solution was observed. The microtiter plates were analysed for microplate readers at 412 nm and results were recorded. Percentage of inhibition calculated based on the mean optical density of enzyme as stated in Equation [1]:

$$\% \text{ Inhibition} = 1 - \frac{\text{Absorbance sample} - \text{Absorbance of background}}{\text{Absorbance blank} - \text{Absorbance of background}} \times 100\% \quad (1)$$

3 | RESULTS AND DISCUSSION

The GC-MS analysis revealed the presence of 13 compounds from the hexane leaf extract of *J. adhatoda* (Figure 2). The major constituents were hexadecanoic acid, methyl ether (RT: 13.55) *n*-hexadecanoic acid (RT: 16.88). phytol (RT: 17.81) along with other minor constituents were also present (Table 2).

Waste materials from agricultural sources are an exceptionally good bio-resource for several products, such as bioethanol, animal feed, cellulose, bio-char and biogenic silica. Groundnut shell, banana peel, coconut husk, orange peel and walnut shell are agro-wastes that are largely constituted of hemicellulose, cellulose, silica, lignin, and trace amount metal ions. Hitherto, many agro-wastes such as rice husk, bamboo leaves waste, sugar beet bagasse etc. have been used for the biogenic synthesis of nano-silica [25–27]. Here, the authors selected rice husk as a source of agro-waste for the biogenic synthesis of nano-silica. Rice husk was pre-treated with different acids (1 N HCl, 1 N HNO_3, 1 N H_2SO_4) and kept in the water bath at 75°C for 30 min to remove ionic and other impurities. The acid pre-treatment removed metal ion impurities and partially hydrolysed the organic substances of these agro-wastes. The incineration

TABLE 2 Phytocomponents identified in the hexane leaf extracts of *J. adhatoda*

RT	Compound name	Molecular formula	Area (%)
.566	3-Pentanol	$C_5H_{12}O_{88}$	0.14
13.55	Hexadecanoic acid, methyl ether	$C_{17}H_{34}O_2$	16.88
16.88	*n*-Hexadecanoic acid	$C_{17}H_{34}O_2$	10.13
17.81	Phytol	$C_{20}H_{40}O$	24.90
23.70	Dotriacontane	$C_{32}H_{66}$	5.6
24.44	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	$C_9H_{12}O_2$	5.03
27.57	4-Pentadecanone	$C_{15}H_{30}O$	1.80
27.26`	Stigmasterol	C29H48O	1.91
28.26	Triisobutyl phosphate	$C_{12}H_{27}O_4P$	6.08
29.69	1 4,5-Dipropenyldihydro-furan-2-one 166	$C_{10}H_{14}O_2$	0.87

TABLE 3 ANOVA for response surface quadratic model (response: synthesis of biogenic nano-silica (mg))

Source	Sum of squares	Df	Mean square	*F*-Value	*p*-Value	
Model	4.91	3	1.64	152.63	< 0.0001	Significant
A-rice husk concentration (g)	0.15	1	0.15	14.18	0.0017	
B-time (h)	1.34	1	1.34	125.26	< 0.0001	
C-calcination temperature (°C)	3.41	1	3.41	318.45	< 0.0001	
Residual	0.71	16	0.011			
Lack of fit	0.12	11	0.011	1.22	0.4403	Not significant
Pure error	0.047	5	9.320E−003			
Cor Total	4.31	19				
Standard deviation	0.0873					
Mean	1.60					
CV%	6.47					
R^2	0.9662					
Adjusted R^2	0.9599					
Predicted R^2	0.9448					
Adequate precision	39.697					

process of the agro-waste after the acid pre-treatment removed organic substances and produced nano-silica with excessive purity and amorphous morphology. This is the simple method and can be appropriate for the large scale production of pure biogenic nano-silica.

A statistical tool response surface methodology through CCD using five-level three variables with six central points was used for improving the synthesis of nano-silica. Maximum synthesis of nano-silica was found to be 2.5 ± 0.10 (mg) at central values of all the factors, namely rice husk concentration (50 g), calcination time (6 h) and calcination temperature (700°C). The Model F-value of 152.63 implies that the model is significant. There is only a 0.01% chance that a 'Model F-Value' of this large could occur due to noise. In this case A, B and C are significant model terms. The 'Lack of Fit F-value' of 1.22 implies the Lack of Fit is not significant relative to the pure error. There is a 44.03% chance that a 'Lack of Fit *F*-value' of this large could occur due to noise. The projected R^2 of 0.9662 is in rational conformity with the adjusted R^2 of 0.9599, that is, the variance is less than 0.033, which implied that the model is significant. The passable precision of 39.697 with a coefficient of variation of 6.47 % endorsed that the model is exceedingly significant and the experiments are accurate, reliable, and the model can be used to pilot the design space. (Table 3) The polynomial equation of second order for the response can be written as

$$R1 = +1.60 - 0.11 \times A + 0.31 \times B + 0.50 \times C \quad (2)$$

where $R1$ was symbolising synthesis of nano-silica (mg) as response and A, B and C were coded terms for the three test

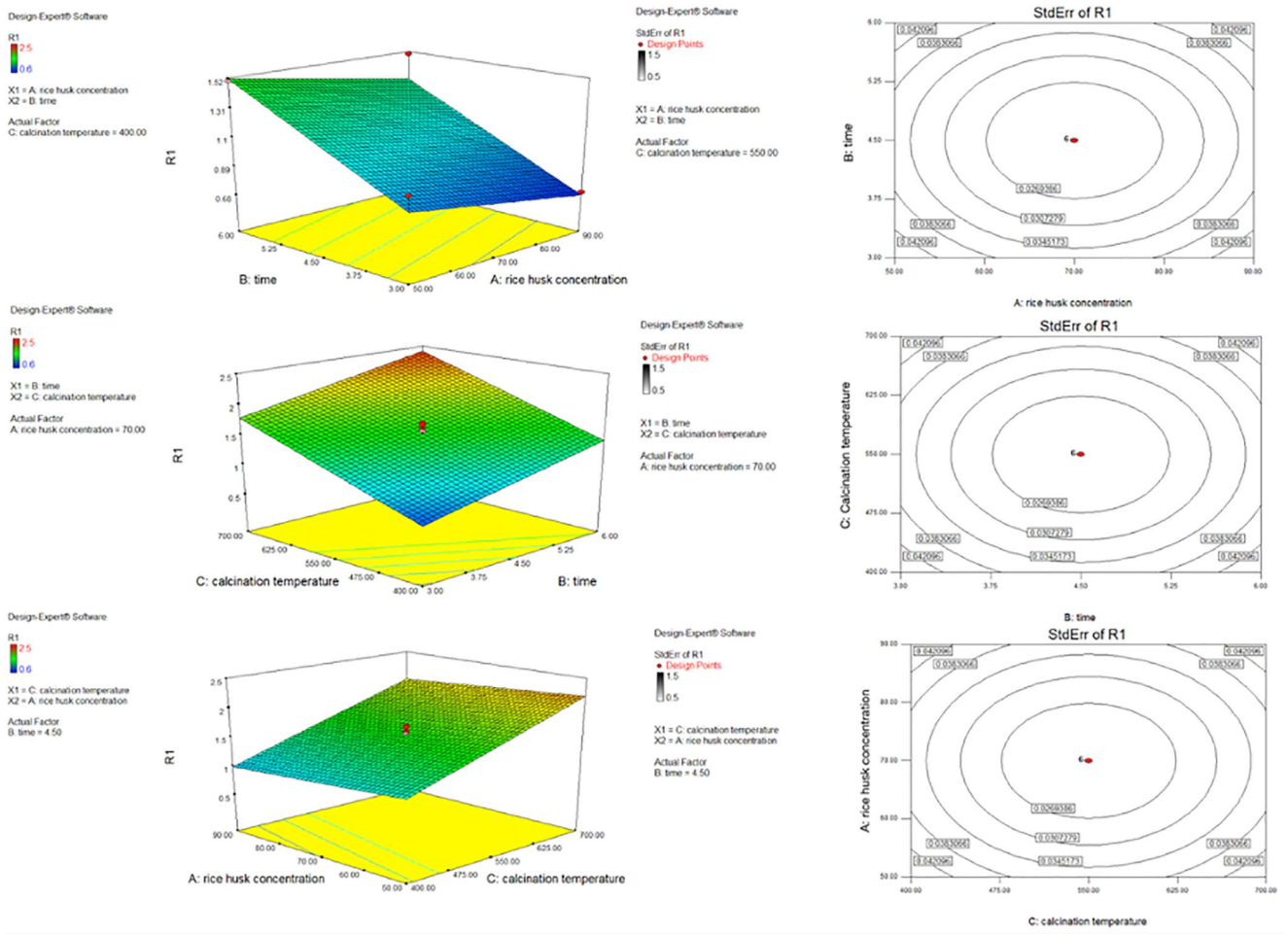

FIGURE 3 Statistical optimisation of parameters for response: Synthesis of nano-silica (mg) using central composite design. 3-D and contour response plots representing the interactions between different variables and its effect on synthesis of silica Nps. (a) interaction between rice husk concentration versus calcination time (AB), (b) interaction between calcination time versus calcination temperature (BC) and (c) interaction between calcination temperature versus rice husk concentration (CA)

variables, namely rice husk concentration, calcination time and calcination temperature, respectively. The 3D interactions between the variables showed a significant influence on the response either independently or in interaction with each other (Figure 3). The model was validated by conducting the synthesis experiment under optimum conditions of all the three variables A, B and C to check the accuracy of the optimisation. The experimental nano-silica synthesis amount was found to be 1.582 ± 0.08 (mg), which was in a favourable concurrence with the prognosticate result 1.97 ± 0.8 (mg) demonstrating the validity of the model as shown in (Table 4). Hence the ideal point prognostication resolved by CCD was efficaciously substantiated, and it was established that it can be used to augment the synthesis parameters of silica. The conventional plot for residuals and predicted versus actual plots were represented respectively (Figure 4). Thus, a significant increase from 0.6 to 2.5 mg in the synthesis of nano-silica were observed under optimised parameters of rice husk 50 g calcination time (6 h) and calcination temperature of (700°C).

3.1 | Characterisation

Figure 5 shows the XRD patterns of silica nanoparticles, *Justicia adhatoda* and synthesised nano-silica: *Justicia adhatoda* conjugate using Cu Kα radiation (λ = 1.5406 Å). Measurements were performed at a voltage of 40 kV and 40 mA within the 2θ range of 10–90°. The synthesised biogenic nano-silica sample exhibited a broad peak at 2θ value of about 22°. The broadness of the exhibited peaks at 22° 2θ confirmed that the synthesised biogenic nano-silica samples were amorphous in nature. The XRD pattern of *Justicia adhatoda* extract showed several characteristic peaks due to its crystalline nature. It can be observed from Figure 5 that *Justicia adhatoda* extract and conjugate retains characteristic peaks at 31°,35° and 39°, which further indicates that conjugate has retained its crystallinity after conjugation and however the intensity of the corresponding peaks has been reduced. Therefore, one can conclude that the *Justicia adhatoda* gets coated on the silica nanoparticles, which is also evident from the SEM and TEM data. Figures 6a,b and 7a,b show SEM and TEM images of

TABLE 4 Actual versus predicted value for response: synthesis of biogenic nano-silica (mg)

Std	Run	Factor 1 A: rice husk concentration (g)	Factor 2 B: Time	Factor 3 C: calcination temperature (°C)	Response (R1) Synthesis of nano-silica
9	1	38.36	4.50	550.00	1.9
14	2	70.00	4.50	802.27	2.4
2	3	90.00	3.00	400.00	0.7
13	4	70.00	4.50	297.73	0.6
19	5	70.00	4.50	550.00	1.67
17	6	70.00	4.50	550.00	1.56
8	7	90.00	6.00	700.00	2.4
12	8	70.00	7.02	550.00	2
18	9	70.00	4.50	550.00	1.5
20	10	70.00	4.50	550.00	1.45
7	11	50.00	6.00	700.00	2.5
15	12	70.00	4.50	550.00	1.7
10	13	103.64	4.50	550.00	1.4
1	14	50.00	3.00	400.00	1
5	15	50.00	3.00	700.00	1.9
6	16	90.00	3.00	706.00	1.7
4	17	90.00	6.00	400.00	1.5
5	18	50.00	6.00	400.00	1.5
11	19	70.00	1.08	550.00	1
16	20	70.00	4.50	550.00	1.6

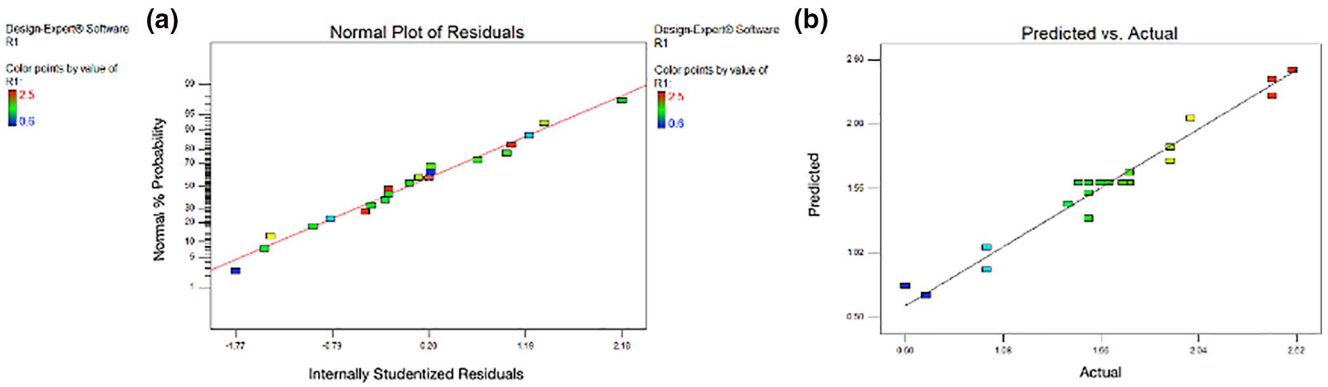

FIGURE 4 ANOVA for response surface quadratic model (response: synthesis of nano-silica). (a) normal plot of residuals and (b) predicted versus actual plot

synthesised silica: *Justicia adhatoda* conjugate. It is observed from these figures that silica nanoparticles are spherical in shape, having diameter of 75 ± 5 nm. It can be seen from these figures that silica nanoparticles are completely surrounded by *Justicia adhatoda* as a shell. The Mastersizer particle analyser was used for the surface area, and pore size analysis of biogenic synthesised nano-silica. Silica: *Justicia adhatoda* conjugate has a larger surface area of 0.986 μm as compared to the biogenic silica nanoparticle with surface area 0.829 μm. The particle size distribution was observed for silica nanoparticle with 50%–90% and it ranged from 566.424 to 911.911 μm, respectively, of silica: *Justicia adhatoda* conjugate (Table 5). Figure 8 shows FTIR spectra of (i) *Justicia adhatoda* (ii) silica nanoparticles and (iii) silica: *Justicia adhatoda* conjugate. Here spectra (i) and (ii) exhibit the typical signatures of both silica nanoparticles and *Justicia adhatoda*. Presence of several absorption bands cm^{-1} indicated the presence of active functional groups at 1392 cm^{-1} (CN bond); 1739, 1684 cm^{-1} (stretching vibration

of C=O); 2802–3113 cm^{-1} (stretching vibration of O–H group) (13*). In spectra (ii) these signatures may be recognised as asymmetric vibration of Si−O (1070.49 cm^{-1}), asymmetric vibration of Si−OH (958.62 cm^{-1}) and symmetric vibration of Si−O (796.60 cm^{-1}) in silica nanoparticles. Spectrum (iii) illustrates the conjugation of *Justicia adhatoda* with silica nanoparticles. This spectrum reveals signatures of both silica nanoparticles and *Justicia adhatoda* molecules. The main phytoconstituents present in the *Adhatoda* plant are *vasicine, vasicinone, vasicol, vasicinol,* vasicinolone etc. [28]. A characteristic band of –NH and –OH groups of extract appeared 3192.19 cm^{-1}. The observation of bands at 2566 and 2867 cm^{-1} are due to the aliphatic C–H stretching frequencies. The presence of carbonyl group and amine of the extract showed the stretching band at 1440 cm^{-1} .The IR band observed at 1020 cm^{-1} is ascribed to aromatic C–H group/heteroatom containing C–C group. The absorption bands appeared in the IR spectrum of extract could also be seen in the IR spectra of *Justicia adhatoda* conjugated silica nanoparticles [29].

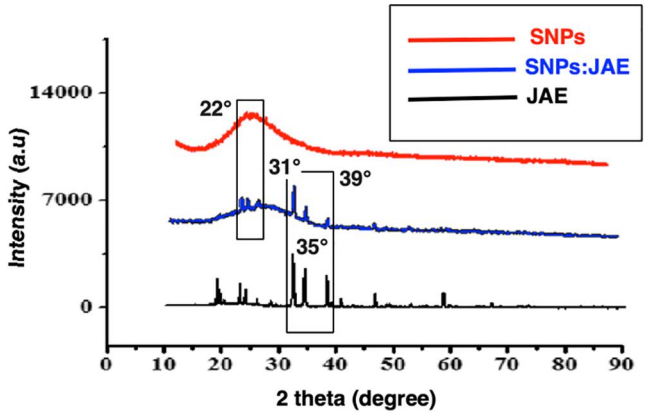

FIGURE 5 XRD patterns of pristine silica: *Justicia adhatoda* (black), silica nanoparticles (red) and silica: *Justicia adhatoda* conjugate (blue)

Thermal analysis of neat silica nanoparticles, *Justicia adhatoda* and silica: *Justicia adhatoda* conjugate was investigated using TGA as shown in Figure 9. It can be observed from this figure that neat silica nanoparticles (red curve) do not exhibit any significant weight loss up to 600°C, except for a small loss at 200−400°C due to the removal of absorbed water. The TGA curve for pure *Justicia adhatoda* (black curve) shows rapid weight loss in the range of 250−445°C, which may be attributed to the degradation of *Justicia adhatoda*. Synthesised silica: *Justicia adhatoda* conjugate also begins to lose weight at 270–300°C with high rate, and afterward it becomes slower. The TGA results show that a ∼40% of lose weight at 270–300°C with high rate, and afterward it becomes slower. The TGA results show that a ∼40% of *Justicia adhatoda* gets loaded on the silica nanoparticles. In vitro release kinetics of *Justicia adhatoda* from silica nanoparticles was studied using UV−vis spectroscopy and is shown in Figure 10. For this purpose, silica: *Justicia adhatoda* conjugate was dispersed (2 mg/mL) in the phosphate buffer solution (PBS: 7.4 pH) and an experiment was performed at 35°C. It is observed from this figure that ∼29% of loaded *Justicia adhatoda* was released within 1 h and then the rate of release becomes slow. Net release of *Justicia adhatoda* was observed up to 50% within 7 h. These results are quite encouraging owing to the agricultural applications of *Justicia adhatoda* as entomotoxic agent of stored grains for longer durations.

Insecticidal activity with treated grains: The toxicity potential of the biogenic silica nanoparticles, silica: *Justicia adhatoda* conjugate (JAC) and *Justicia adhatoda* extract (JAE) *S. oryzae*, are presented in Figure 10. Based on the results obtained, the silica: *Justicia adhatoda* conjugate exhibited the highest percentage of mortality for storage pest at a concentration level of 1mg of conjugate with 5gm of rice treated over an exposure period of 10 days (Figure 11).

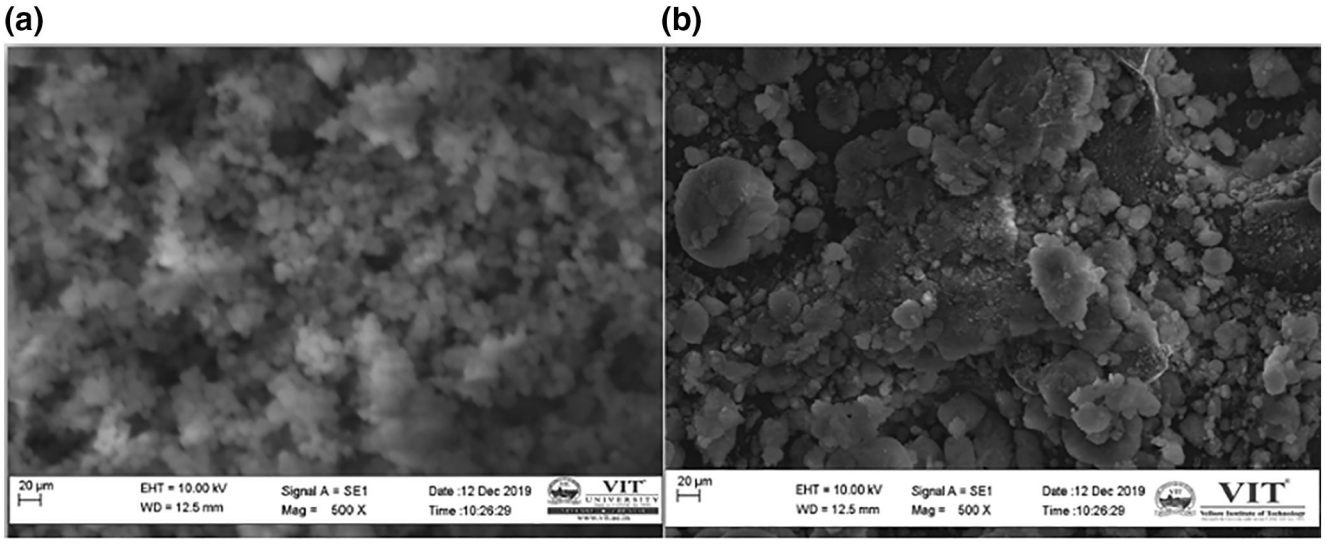

FIGURE 6 SEM images of silica nanoparticles (A) and *Justicia adhatoda* conjugate (B)

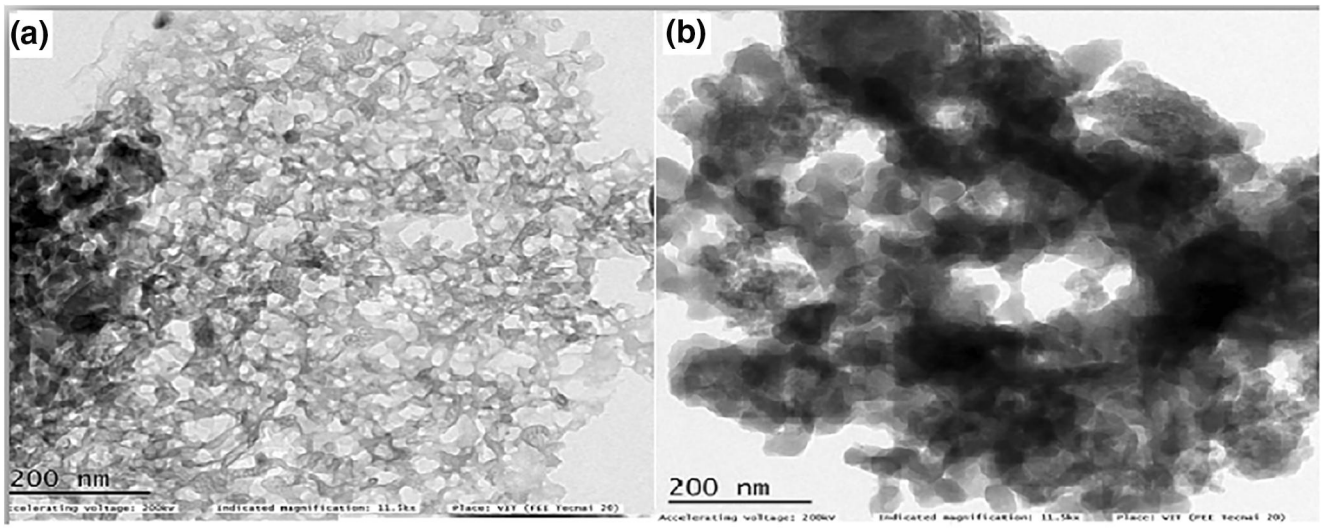

FIGURE 7 TEM images of (A) silica nanoparticles and (B) *Justicia adhatoda* conjugate

TABLE 5 Mastersizer particle analysis of biogenic silica nanoparticles and silica: *Justicia adhatoda* conjugate

Sample	Specific surface Area m^2/g	*d*(0.5): um	*d*(0.9): um
Biogenic silica nanoparticles	0.829 ± 0.9	566.424 ± 9.6	883.778 ± 1.7
Justicia adhatoda conjugate	0.986 ± 0.8	806.671 ± 2.9	911.911 ± 3.8

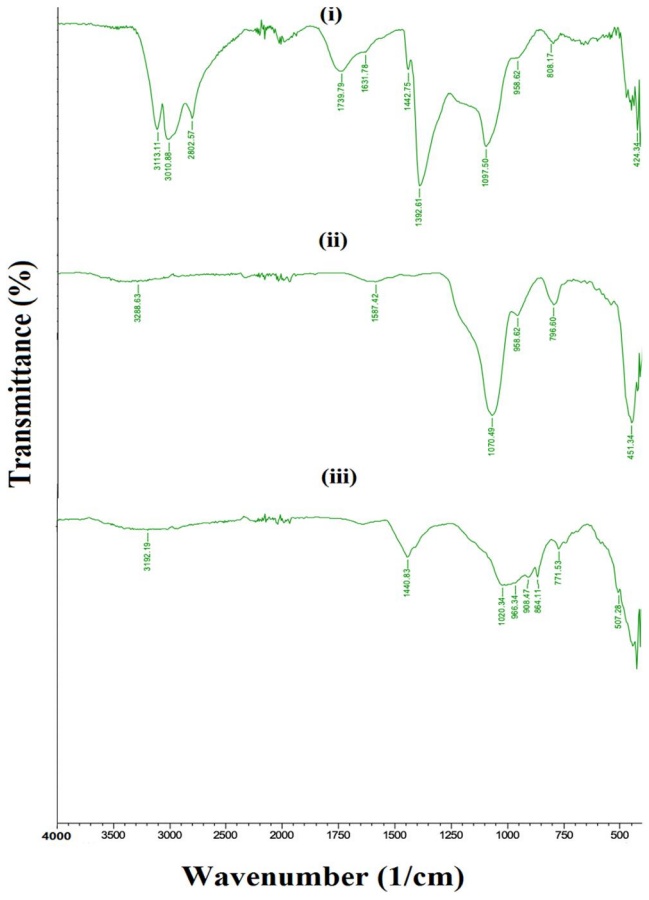

FIGURE 8 FTIR spectra of (i) *Justicia adhatoda,* (ii) silica nanoparticles and (iii) silica: *Justicia adhatoda* conjugate

Cholinesterase enzymes inhibitory activity: The initial AChE inhibition activity of the three all the three samples (biogenic silica nanoparticles, silica: *Justicia adhatoda* conjugate (JAC) and *Justicia adhatoda* extracts) was evaluated on the enzyme supernate extract from insect. Silica: *Justicia adhatoda* conjugate (JAC) and *Justicia adhatoda* extracts showed potent AChE inhibition for insects. Silica: *Justicia adhatodaa* conjugate (JAC) and *Justicia adhatoda* extracts showed high AChE inhibition on enzyme supernate extracted from *S. oryzae*. The silica nanoparticles showed almost negligible AChE inhibition (Figure 12).

4 | CONCLUSIONS

Justicia adhatoda loaded biogenic silica nanoparticles epitomises a successful green and non-bio accumulating slow release biocide system. *Justicia adhatoda* compounds were covalently bonded to the biogenic silica support and it was possible to increase their stability in water and to promote higher thermal stability as well. In conclusion, we have conjugated *Justicia adhatoda* with silica nanoparticles by using a simple wet chemical method. Synthesised silica: *Justicia adhatoda* conjugate was well dispersed and stable in aqueous medium at room temperature. Nearly 40% of *Justicia adhatoda* was getting loaded on silica as observed from TGA analysis. The inhibition of AChE may have direct effect on bradycardia, bronchoconstriction and prolonged muscle contraction of insects that lead to paralysation and death. The biochemical assay revealed the possible connection between the AChE inhibition and the toxicity.

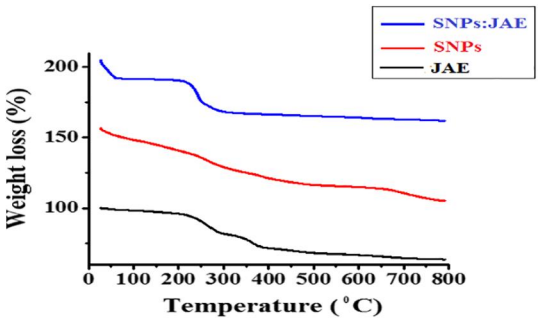

FIGURE 9 TGA curves for silica nanoparticles (red), *Justicia adhatoda* extract (black) and silica: *Justicia adhatoda* conjugate (blue)

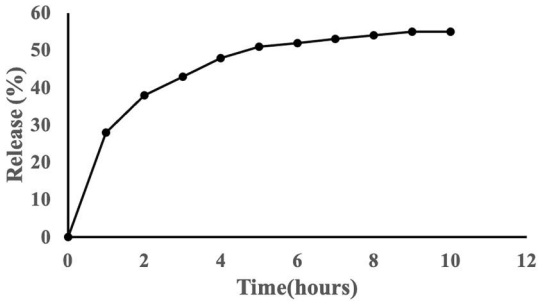

FIGURE 10 Graph of in vitro release kinetics of *Justicia adhatoda* at 35°C in buffer solution (pH = 7.4)

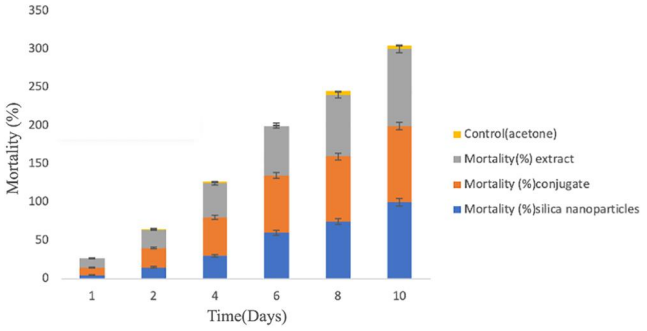

FIGURE 11 Mean mortality percentage of *S. oryzae*, on rice grain treated with silica nanoparticles, *Justicia adhatoda* extract and silica: *Justicia adhatoda* conjugate

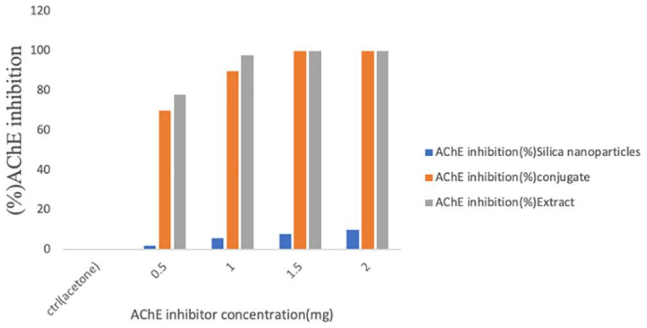

FIGURE 12 Inhibition on acetylcholinesterase enzyme by silica nanoparticles, *Justicia adhatoda extra*ct and silica: *Justicia adhatoda* conjugate

ACKNOWLEDGEMENTS

Authors are thankful to School of Advanced Sciences (SAS), School of Bio-Medical Sciences (SBST) and DST-FIST/VIT SEM of Vellore Institute of Technology, India, for providing laboratory facilities for conducting the experimental work.

The authors state that they haven't received any funds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

ORCID

Peerzada Gh Jeelani https://orcid.org/0000-0001-7979-3303
C Ramalingam https://orcid.org/0000-0001-6517-4536

REFERENCES

1. Thanigaivel, A., et al.: Chemicals isolated from *Justicia adhatoda* Linn reduce fitness of the mosquito, Aedes aegypti L. Arch. Insect Biochem. Physiol. 94(4), e21384 (2017)
2. Iqbal Chowdhury, I., et al.: Supplements of an aqueous combination of *Justicia adhatoda* and *Ocimum tenuiflorum* boost antioxidative effects and impede hyperlipidemia. Animal Model. Exp. Med. 3(2), 140–151 (2020)
3. Mattos, B.D., Rojas, O.J., Magalhães, W.L.: Biogenic silica nanoparticles loaded with neem bark extract as green, slow-release biocide. J. Clean. Prod. 142, 4206–4213 (2017)
4. Koehler, P.G.: Rice Weevil, *Sitophilus oryzae* (Coleoptera Curculionidae). University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS (1999)
5. Struelens, Q., Silvie, P.: Orienting insecticide research in the tropics to meet the sustainable development goals. Curr. Opin. Insect Sci. 40, 24–30 (2020)
6. Daglish, G.J., et al.: Effects of time and concentration on mortality of phosphine-resistant *Sitophilus oryzae* (L.) fumigated with phosphine. Pest. Manage. Sci. 58, 1015–1021 (2002)
7. Debnath, N., et al.: Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). J. Pest Sci. 84(1), 99–105 (2011)
8. Rani, P.U., Madhusudhanamurthy, J., Sreedhar, B.: Dynamic adsorption of α-pinene and linalool on silica nanoparticles for enhanced antifeedant activity against agricultural pests. J. Pest Sci. 87(1), 191–200 (2014)
9. Rajendran, S.: Responses of phosphine-resistant strains of two stored-product insect pests to changing concentrations of phosphine. Pest. Sci. 40(3), 183–186 (1994)
10. Benhalima, H., et al.: Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Product. Res. 40(3), 241–249 (2004)
11. Heather, N.W.: Sex-linked resistance to pyrethroids in *Sitophilus oryzae* (L.)(Coleoptera: Curculionidae). J. Stored Product. Res. 22(1), 15–20 (1986)
12. Stathers, T.E., Denniff, M., Golob, P.: The efficacy and persistence of diatomaceous earths admixed with commodity against four tropical stored product beetle pests. J. Stored Product. Res. 40(1), 113–123 (2004)
13. Roy, A., et al.: Controlled pesticide release from biodegradable polymers. Cent. Eur. J. Chem. 12, 453e469 (2014) http://dx.doi.org/10.2478/s11532-013-0405-2
14. Battegazzore, D., et al.: Rice husk as bio-source of silica: preparation and characterization of PLA–silica bio-composites. RSC Adv. 4(97), 54703–54712 (2014)
15. Jeelani, P.G., et al.: Multifaceted application of silica nanoparticles. Rev. Silicon. 12(6), 1337–1354 (2020)
16. Thuc, C.N.H., Ha Thuc, H.: Synthesis of silica nanoparticles from Vietnamese rice husk by sol–gel method. Nanoscale Res. Lett. 8, 1–10 (2013)
17. Boonmee, A., Jarukumjorn, K.: Preparation and characterization of silica nanoparticles from sugarcane bagasse ash for using as a filler in natural rubber composites. Polym. Bull. 77(7), 3457–3472 (2020)

18. Peerzada, J.G., Chidambaram, R.: A statistical approach for biogenic synthesis of nano-silica from different agro-wastes. Silicon, 1–13 (2020). https://doi.org/10.1007/s12633-020-00629-5
19. Song, S., Cho, H.B., Kim, H.T.: Surfactant-free synthesis of high surface area silica nanoparticles derived from rice husks by employing the Taguchi approach. J. Indus. Eng. Chem. 61, 281–287 (2018)
20. Gangwar, R.K., et al.: Curcumin conjugated silica nanoparticles for improving bioavailability and its anticancer applications. J. Agri. Food Chem. 61(40), 9632–9637 (2013)
21. Manju, S., Sreenivasan, K.: Gold nanoparticles generated and stabilized by water soluble curcumin−polymer conjugate: lood compatibility evaluation and targeted drug delivery onto cancer cells. J. Colloid Interf. Sci. 368, 144−151 (2012)
22. Batchelor, L., et al.: Manufacture of mesoporous silicon from living plants and agricultural waste: an environmentally friendly and scalable process. Silicon. 4(4), 259–266 (2012)
23. Ahmad, S., et al.: A phyto-pharmacological overview on *Adhatoda zeylanica* Medic. syn. *A. vasica* (Linn.) Nees. Nat. Product Radiance. 8, 549–554 (2009)
24. Hematpoor, A., et al.: Insecticidal activity and the mechanism of action of three phenylpropanoids isolated from the roots of Piper sarmentosum Roxb. Sci. Report. 7(1), 1–13 (2017)
25. Grisdanurak, N., Chiarakorn, S., Wittayakun, J.: Utilization of mesoporous molecular sieves synthesized from natural source rice husk silica to chlorinated volatile organic compounds (CVOCs) adsorption. Korean J. Chem. Eng. 20(5), 950–955 (2003)
26. Wang, W., Martin, J.C.: Xiaotian Fan, Aijie Han, Zhiping Luo, and Luyi Sun. "Silica nanoparticles and frameworks from rice husk biomass. ACS Appl. Mater. Interf. 4(2), 977–981 (2012)
27. Nalan, O.S., et al.: Novel onstep synthesis of silica nanoparticles from sugar beet bagasse by laser ablation and their effects on the growth of fresh water algae culture. Particulogy. 17, 729–735 (2014)
28. Sajeeb, B. K., et al.: Evaluation of *Justicia adhatoda* L. syn. *Adhatoda vasica* Nees extract by major metabolite analysis (2015)
29. Ponvel, K., Narayanaraja, T., Prabakaran, J.: Biosynthesis of Silver nanoparticles using root extract of the medicinal plant *Justicia adhatoda*: Characterization, electrochemical behavior and applications. Intl. J. Nano Dimension. 6(4), 339–349 (2015) https://doi.org/10.7508/ijnd.2015.04.002

How to cite this article: Jeelani PG, Ramalingam C. Statistical approach to synthesise biogenic silica nanoparticles from rice husk and conjugated with *Justicia adhatoda* extract as green, slow-release biocide. *IET Nanobiotechnol*. 2021;15:391−401. https://doi.org/10.1049/nbt2.12027