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Optimization studies on biodegradation of atrazine by *Bacillus badius* ABP6 strain using response surface methodology

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ABSTRACT

In this study, the optimization of distinctive environmental factors such as pH, temperature, agitation-speed and atrazine-concentration on atrazine degradation by utilizing *Bacillus badius* ABP6 strain, has been done through response-surface-methodology (RSM). The optimum-conditions after analysis for the maximum atrazine degradation were: pH 7.05, temperature 30.4 °C, agitation-speed 145.7 rpm, and atrazine-concentration 200.9 ppm. The prescribed model was approved for high F-value (95.92), very low P-value (<0.01) and non- significant lack of fit (0.1627). It was observed that under the optimized-conditions, the R^2 value of regression models for all the response variables was 0.9897 and the maximum atrazine degradation i.e. 89.7 % was found. Finally for graphical representation, the validated optimum-conditions of variables and responses were simulated using three dimensional plots (3D). The confirmation of the model is successful to suggest the optimization parameters of atrazine degradation under in-situ condition by bacterial isolate employing response-surface-methodology optimization tool of Design expert software (new version 10.0.1).

1. Introduction

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)- 1,3,5-triazine], a derivative of the S-triazines is the most extensively used herbicide in agricultural and forestry sectors. Annually 70,000–90,000 tonnes range of atrazine is used to control grassy weeds and broadleaf weeds in crops like maize, sugarcane and sorghum [1]. Atrazine herbicide is most common organic pollutant of surface water, ground water, and soil because of its low biodegradability [2]. Various toxic effects of atrazine were also observed on fishes, algae, insects, aquatic plants, and mammals [3,4]. Amongst the prevalent physical, chemical, as well as biological methods to remove atrazine from soil and water [5], the biological processes have advantages over others [6–8]. As compare to other traditional strategies, this method is cost-effective, environment friendly, permanent and non invasive to natural ecosystem [9,10]. Consequently, the biodegradation of hazardous chemicals, at environmentally pertinent concentrations attracts inquisitiveness in both academia and industry [11]. Previously biodegradation of atrazine was reported in literature [12–14] but very few reports are available on isolation with characterization of specific atrazine degrading bacteria and their optimization studies in biodegradation of atrazine. Hence, present study is focused on optimization in context with atrazine degradation by soil bacterial isolate of atrazine contaminated site using new Design expert software (version 10.0.1).

Response-surface-methodology (RSM) is an important tool use for building models, statistically designing experiments, and to evaluate the effects of different factors for searching suitable optimum-conditions. The usage of this method is in lots of fields such as environmental engineering, food technology and biotechnology, respectively [15–19]. It is prominent statistical technique to examine the interactive effects among numerous factors at diverse levels, effectively used for the optimization of pollutants removal rate [20–22]. As compared to other processes of optimization, RSM has several advantages like it saves energy, time and resources by reducing the quantity of experimental runs needed to evaluate multiple parameters with less difficulty [23]. The Polynomial model is used to fit the actual responses with variety of optimal responses and determines relationship among variables and responses [24]. The design known as Box–Behnken design (BBD) is a type of response surface methodology that is rotatable and requires 3 levels of each factor. This design is more efficient, widely used in many studies and easier to systematize experiment as compare to others. The independent quadratic design i.e. BBD, does not contain midpoints of edges and fractional factorial design with the treatment combinations [25–27]. Researchers has been proposed RSM to optimize various

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parameters for chemical pollutants/pesticides bioremediation [28–32]. There is no information in the literature about atrazine degradation with Bacillus sp. ABP6 and optimization of degradation parameters with RSM.

Therefore, the aim of present study was to isolate and characterize atrazine degrading bacteria with optimizing conditions. This study reports atrazine degradation by *Bacillus badius* for the first time. Besides this, RSM based on the Box–Behnken design was used to determine the optimum environmental conditions for growth and atrazine degradation by *Bacillus badius* ABP6 strain with new version (10.0.1) of Design expert software-RSM tool. This bacterial isolate of *Bacillus badius* ABP6 strain was recovered from the pesticide contaminated soil of tarai agro-ecosystem of Uttarakhand, able to degrade atrazine more efficiently. This paper also highlights potential use of *Bacillus badius* ABP6 strain for the remediation of atrazine under in-situ condition.

2. Materials and methods

2.1. Chemicals and media

Atrazine (>98 % purity) and chromatographic grade-methanol was procured from Sigma-Aldrich, USA. Atrazine was dissolved in methanol, to make stock solution of 100 mg/L and then stored at 4 °C prior to use. Minimal salts medium (g L^{-1}) used for the isolation purpose contained the following: KH_2PO_4, 1.0; K_2HPO_4, 1.0; $MgSO_4$ 0.2; NH_4NO_3 1.0; Fe (SO_4) 0.01; $CaCl_2$ 0.02, and composition of Nutrient agar medium (g L^{-1}) was: Peptone 10; Yeast-extract 5.0, agar 20.0, NaCl 5.0, pH 7.0 and was sterilized at 121 °C for 20 min [33].

2.2. Enrichment and isolation of atrazine degrading bacterium

Atrazine contaminated soil-samples (0–15 cm), were collected from Norman E. Borlaug, Crop Research-Centre, G.B.P.U.A.&T., Pantnagar, after 10 days of herbicide spray in the Maize fields. Atrazine degrading bacterial strain ABP6 was isolated from contaminated soil using the enrichment method [34]. In order to isolate herbicide degrading microorganisms, 5 g of soil sample was inoculated in 250 mL Erlenmeyer flask with 50 mL of sterilized minimal salts medium containing 50 mg/L atrazine. The enrichment-culture was incubated in a shaking incubator, at 30 °C and 150 rpm. After 7 days, 5 mL of broth culture, from each flask were reinoculated to 50 mL of fresh media containing 100 mg/L atrazine and were cultured under the same conditions. Then, the same procedure, was repeated twice upto-200 mg/L concentration of atrazine. Finally, 0.2 mL of final culture broth was pour-plated on nutrient agar to isolate bacterium colony. Each discrete colony was considered as a different-species, was repeatedly-streaked on agar plates. Pure cultures were obtained from each individual colony by further repeated streaking. The atrazine degrading ability of purified isolates was determined by minimum inhibitory concentration method based on growth inhibition by atrazine. The isolated bacterium was gram positive and rod shaped *Bacillus sp.* with morphology and the most active strain was identified by 16S rDNA sequencing analysis.

2.3. 16S rRNA analysis and construction of phylogenetic-tree

After isolation process the bacterium was encoded as ABP6 strain and selected based on its highest ability for atrazine degradation. Then investigated for morphological and biochemical characteristics by API 20 NE system (Analytical Profile Index, France). Bacterial isolate was distinguished by using partial sequencing of 16S rRNA. PCR was performed under set conditions such as: 30 cycles of denaturation at 94 °C (1 min), annealing at 55 °C (1 min) and extension at 72 °C (1 min). PCR products were purified and sequenced by Chromus Biotech Ltd. Banglore, India. Identification was carried out on the basis of 16S rRNA homology between the query and reference sequences available at GenBank using BLAST algorithm of the National Centre for Biotechnology Information (NCBI) database at www.ncbi.nlm.nih.gov/BLAST. Phylogenetic analysis was done using the neighbor-joining method with bootstrap values calculated from 1000 replicates run, with MEGA version 7.0 software packages.

2.4. Experimental-process

Table 1, represents the range of independent-variables and levels. Experiments were carried out according to the Box–Behnken design, shown in Table 2. As per the requirement of Box Behnken design, three levels of treatments were employed for atrazine-concentration (150, 200 and 250 ppm), agitation-speed (100,150, 200 rpm) using an incubator shaker), temperature (20, 30 and 40 °C) and pH (5, 7, 9). Then, the degradation ability of bacteria was investigated by inoculating the bacterial culture into different Erlenmeyer flasks (250 mL) containing minimal salt medium (50 mL) with above mentioned optimized conditions and placing them into an incubator shaker. After that, the amounts of atrazine residues after biodegradation were determined at different time interval from 0 days to 20 days by HPLC.

2.5. Residual atrazine analysis

After incubation, atrazine residue was extracted from sample by liquid-extraction method. 5 ml aliquots were taken from broth culture and centrifuged to obtain cell-free supernatant. 5 ml of dichloromethane was added to the solution and shaken vigorously for 3 min. The whole content was allowed to stand-quiet, to separate the water and dichloromethane-layer. Above process was repeated two times with the aqueous phase by addition 5 ml dichloromethane each time. The organic phases of all the three extractions were pooled. The separated extracts were dried over, anhydrous-Na_2SO_4 and the volume was measured. Final sample was taken with methanol and the residual atrazine was analyzed

Table 1
Levels of different process variables in coded and uncoded form for atrazine degradation.

Variables	Code	Levels		
		−1	0	1
pH	X_1	5	7	9
Temperature (°C)	X_2	20	30	40
Agitation-speed (rpm)	X_3	100	150	200
Atrazine-concentration (ppm)	X_4	150	200	250

Table 2
Experimental conditions of Box Behnken design for atrazine degradation.

Exp. Run No.	pH	Temperature (°C)	Agitation speed (rpm)	Atrazine conc. (ppm)	% Atrazine degradation	
					Experimental (Actual)	Predicted
1	−1	0	0	−1	73.5	74.1
2	0	1	0	−1	75.3	76.4
3	−1	0	1	0	58.9	60.4
4	1	1	0	0	54.7	54.5
5	0	−1	0	1	69.8	69.5
6	0	−1	1	0	61.2	60.8
7	0	0	0	0	89.6	88.6
8	0	0	1	−1	64.5	64.3
9	0	−1	−1	0	77.6	78.4
10	1	0	0	−1	75.7	73.8
11	0	0	0	0	88.9	88.6
12	0	0	0	0	87.6	88.6
13	1	0	1	0	50.3	51.9
14	−1	0	0	1	72.1	74.2
15	0	1	0	1	63.4	64.8
16	0	1	−1	0	81.0	81.7
17	0	0	0	0	87.3	88.6
18	0	0	0	0	89.7	88.6
19	0	0	−1	1	79.3	78.3
20	−1	−1	0	0	65.9	64.8
21	1	0	−1	0	74.8	74.1
22	1	−1	0	0	69.4	70.7
23	−1	0	−1	0	83.8	83.0
24	0	0	−1	−1	86.5	87.3
25	1	0	0	1	57.2	56.9
26	0	0	1	1	58.7	56.6
27	0	−1	0	−1	75.2	74.6
28	0	1	1	0	55.2	54.6
29	−1	1	0	0	80.6	78.0

on a Dionex ultimate 3000 HPLC equipped with a C18 reversed phase column (250 × 4.6 mm id, 5 μm). All the experiments were completed in three independent experiments, and the results were the means of three replicates.

The degradation rate of atrazine was analyzed according to the following equation (Eq. (1)):

$$\%\text{degradation}\frac{A_s}{A_0} \times 100 \quad (1)$$

Where, A (mg/L) is the residual concentration of sample, and A0 (mg/L) represents the concentration of control sample.

2.6. Design of experiment by RSM

RSM based on the Box–Behnken design is an experiential statistical technique used to investigate the influence of interactive effects of the selected parameters on atrazine degradation by strain ABP6. This method is employed for multiple regression analysis by quantitative data obtained from appropriately deliberated experiments. A Box-Behnken design was performed in order to study the effects of different variables towards their responses for optimization on atrazine biodegradation by single-factor experiments and also suitable for fitting a quadratic surface model.

In the optimization study, BBD was used to determine three level four factor test. pH (X_1), temperature (X_2), agitation-speed (X_3) and atrazine-concentration (X_4) were selected as Independent variables for the optimization studies of atrazine degradation. Low and high levels of each variable were coded as: -1 and+1 by keeping 0 as mid-point. Levels of different process variables in coded and uncoded form are represented in Table 1 and the experimental and predicted values of degradation by a complete three-factor-three level factorial experimental design, with three replications at the central points, are represented in Table 2. For minimizing the effects of unexplained variability and systematic errors in the observed responses, all the experiments were conceded randomly. Twenty-nine runs were used for optimizing the range and levels of chosen variables. Second-order polynomial equation was assumed for predicted response and the coded-values of the process-parameters were calculated with the help of following-equation given by Eremia [35]:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \times 100 \quad (2)$$

Where, x_i = coded-value of the i^{th} variable, X_i = uncoded-value of the i^{th} test variable, X_0 = uncoded-value of the i^{th} test variable at center-point and ΔX_i = range of values with the step-change.

Researchers used mathematical-quadratic-model and analysis of variance (ANOVA) for data testing [36]. Since various responses were the results of interactions of independent variables therefore, the following polynomial regression-equation was taken for the explanation of performance of the model:

$$Y = A_0 + \sum_{j=1}^{k} A_j X_j + \sum_{j=1}^{k} A_{ii} X_j^2 + \sum_{x-1}^{j=1} \sum_{j=2}^{k} A_{ij} X_i X_j + \in \quad (3)$$

Where, Y = predicted response (dependent variable), A_0 = constant, A_{ij} = interaction coefficient, A_{ii} = squared coefficient, X_i and X_j are the levels of independent variables and ε is error. They represent the linear, quadratic, and cross product effects of the X_1, X_2, X_3 and X_4 factors on the response, respectively. The model was acquired for the assessment of each independent variable effects towards the response i.e. degradation [35,37–39]. It was also observed that for the calculation of F-value, the uppermost order model with significant conditions was usually selected. The effect of the independent variable is considered significant, when F-value being calculated from the data was greater than theoretical-value [40]. Various preliminary experiments were performed to determine the extreme-values of the variables. New Design Expert software (version 10.0.1, Stat-Ease, Inc., Minneapolis, USA) was used to

execute the regression as well as graphical analysis with statistical significance. Using RSM, the three-dimensional response surface plots of atrazine degradation were presented as a function of independent variables.

2.7. Statistical analysis

The experimental data were processed for calculating standard error of means and multi-factorial analysis of variance (ANOVA) as available in the SPSS statistical package (Statgraphics Plus V. 11), and expressed at 0.05 probability level.

3. Results

3.1. Identification of atrazine degrading bacterium

An indigenous bacterium capable of utilizing atrazine as a sole carbon and nitrogen source was eventually isolated from the atrazine contaminated soil. Bacterial strain ABP6 was a gram positive, rod shaped bacterium, spore forming and showed positive enzymatic reactions for oxidase, catalase with assimilation of Lactose, Dextrose, fructose, trehalose and with other carbohydrates (Table 3). The 16S rRNA gene Sequence was deposited in the Gene Bank database under accession number MG680921. Homology search using BLAST revealed 97 % similarity of this sequence of strain ABP6 with 16S rRNA gene sequence of *Bacillus badius strain* NBRC 15713 (BCVF01000097.1) (GeneBank accession no. LWDO01000012.1), giving the phylogenetic relationship of this bacterial isolate with *Bacillus badius strain* NBRC 15713 (Fig. 1), thus isolate was designated to be *Bacillus badius* ABP6 strain (MG680921).

Table 3
Morphological and Biochemical characteristics for atrazine degrading strain ABP6.

Characterstics	*Bacillus badius* ABP6 strain
Morphological	
Colony color	Creamy white
Gram nature	positive
Cell morphology	Rod shaped
Biochemical	
Lactose	+
Xylose	–
Maltose	–
Fructose	+
Dextrose	+
Galactose	–
Raffinose	–
Trehalose	+
Melibiose	–
Sucrose	+
L-Arabinose	–
Mannose	–
Catalase	+
Inulin	+
Sodium gluconate	–
Glycerol	–
Salicin	+
Glucosamine	+
Dulcitol	+
Inocitol	+
Sorbitol	+
Mannitol	+
Adonitol	+
a-Methyl-D-glucoside	+
Ribose	–
Oxidase	+

3.2. Optimization of atrazine degrading conditions by Bacillus badius ABP6 strain

Experiments were completed to scrutinize the combined effect of four different process parameters on atrazine degradation using bacterial isolate of *Bacillus badius* ABP6 strain. The Design Expert (10.0.1) software was used to determine the second order polynomial coefficients for each term of the equation through multiple-regression analysis. All the experimental and predicted values of % atrazine degradation by bacterial isolate are specified in Table 1. The maximum degradation rate was observed in Experiment 18 with pH 7($X_1 = 0$),Temperature 30 ($X_2 = 0$), Agitation-speed 150 rpm ($X_3 = 0$) and Atrazine-concentration 200 ppm ($X_4 = 0$), while the minimum degradation rate was observed in experiment 25 with pH 9($X_1 = 1$),Temperature 30 ($X_2 = 0$), Agitation-speed 150 rpm ($X_3 = 0$) and Atrazine-concentration 250 ppm ($X_4 = 1$). It was examined that as the pH increased the degradation rate decreased, indicating that neutral pH was suitable for biodegradation. Increased temperature and agitation-speed resulted as increased rate of degradation, whilst increased atrazine-concentration reduced its degradation-rate.

3.3. Numerical analysis on atrazine degradation

3.3.1. Effects of influencing factors on atrazine degradation

It was shown in the Table 2 that all levels like linear, quardratic and interaction effecting the influencing factors. It is revealed from the table that the effects on degradation rate of all factors at linear and quadratic level were found to be highly-significant ($p < 0.01$). The effect of individual influencing factors (pH, Temperature, Agitation-speed and Atrazine-concentration) on atrazine degradation was computed using sequential sum of square. Results shows that agitation-speed was highly significant at 1% level of significance ($p < 0.01$) followed by pH.

3.3.2. Fitting of quadratic-model and design of experiment

It was represented by the second order polynomial Eq. (4), that the mathematical-model relating the percentage degradation using bacterial isolate of contaminated field soil with independent process variables.

$$Y = 88.62 - 4.39X_1 - 0.74X_2 - 11.18X_3 - 4.18X_4 - 7.35X_1X_2 + 0.100X_1X_3 - 4.27X_1X_4 - 2.35X_2X_3 - 1.62X_2X_4 + 0.35X_3X_4 - 11.56X_1^2 - 10.01X_2^2 - 9.69X_3^2 - 7.27X_4^2 \quad (4)$$

Significant predictive-equation for degradation rate (%) is given below:

$$Y = 88.62 - 4.39X_1 - 11.18X_3 - 4.18X_4 - 7.35X_1X_2 - 4.27X_1X_4 - 2.35X_2X_3 - 11.56X_1^2 - 10.01X_2^2 - 9.69X_3^2 - 7.27X_4^2 \quad (5)$$

Where, Y is the % degradation, X_1, X_2, X_3, and X_4 are the coded values of the test variables, pH, temperature (°C), agitation-speed (rpm) and Atrazine-concentration (ppm), respectively. The above mathematical-model can be used to predict the % degradation of atrazine with different experimental conditions. Fig. 2 represents the validation of the predicted response values with actual response values.

3.3.3. Analysis of variance for response surface quadratic model

Analysis of variance (ANOVA) is required to test the significance and adequacy of the quadratic response surface model. A quadratic model was built to predict the degradation efficiency and ANOVA was used to determine the significance and interactions of the variables. All the observation with ANOVA for atrazine is presented in Table 4 and in which determination coefficient (R^2) indicated that maximum responses were covered by the model. It was also

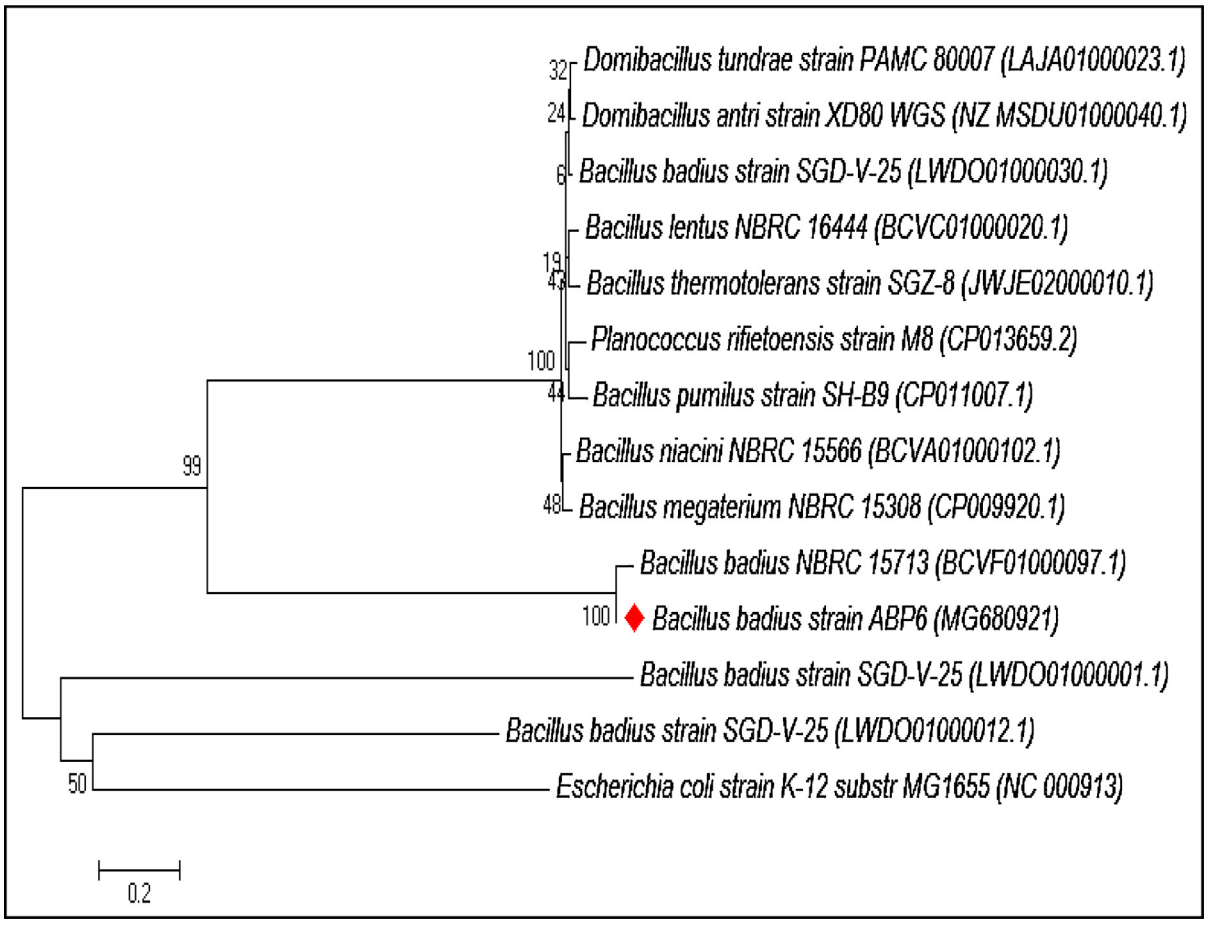

Fig. 1. Phylogenetic tree of *Bacillus badius* strain ABP6 (MG680921) constructed by neighbor-joining method based on nucleotide sequences of the partial 16S rRNA genes. The numbers at the nodes represents percentage bootstrap values for 1000 replicates run.

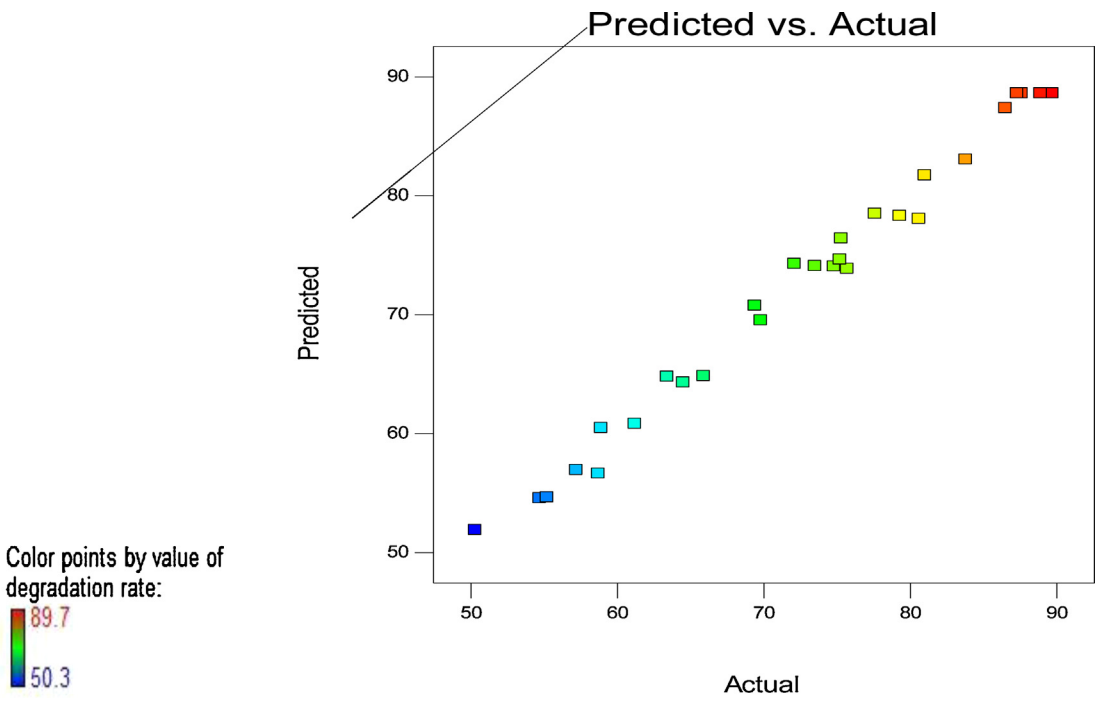

Fig. 2. Validation of Predicted vs. Actual values of atrazine degradation.

Table 4
Analysis of variance for response surface quadratic model.

Source	Coefficient factor	Sum of square	Degree of freedom	Mean square	F-value	P- value Prob > F	
Model	88.62	3881.90	14	277.28	95.92	<0.0001	Significant
X_1	4.39	231.44	1	231.44	80.06	<0.0001	
X_2	0.74	6.60	1	6.60	2.28	0.1530	
X_3	11.18	1500.80	1	1500.80	519.17	<0.0001	
X_4	4.18	210.00	1	210.00	72.65	<0.0001	
X_1X_2	7.35	216.09	1	216.09	74.75	<0.0001	
X_1X_3	0.1	0.040	1	0.040	0.014	0.9080	
X_1X_4	4.27	73.10	1	73.10	25.29	0.0002	
X_2X_3	2.35	22.09	1	22.09	7.64	0.0152	
X_2X_4	1.62	10.56	1	10.56	3.65	0.0766	
X_3X_4	0.35	0.49	1	0.49	0.17	0.6868	
X_1^2	11.56	866.19	1	866.19	299.64	<0.0001	
X_2^2	10.01	649.41	1	649.41	224.65	<0.0001	
X_3^2	9.69	609.47	1	609.47	210.84	<0.0001	
X_4^2	7.27	342.67	1	342.67	118.54	<0.0001	
Residual		40.47	14	2.89			
Lack of fit		35.48	10	3.55	2.85	0.1627	Non Significant
Pure error		4.99	4	1.25			
Cor total		3922.37	28				

R^2 = 0.9897, Adj- R^2 = 0.9794.

demonstrated that with the experimental values, the predicted values were in good concurrence. The model for atrazine biodegradation is highly significant ($p < 0.0001$), indicating that the established quadratic model for atrazine degradation by bacterial isolates was adequate and reliable in representing the actual relationship between responses and variables. The F-ratio is the suitable measure to illustrate the variables differentiation in the data with reference to its mean. The significant F-value (95.92) and the non-significant lack of fit (0.1627) value through the ANOVA of the data imply that the quadratic model is highly significant (Table 4). Calculated F-value, from the data (95.92) was greater than tabulated-value, which indicates that the factors adequately explain the dissimilarity in the data and also implies that this model is significant and the estimated-factors are real. Values of $P < 0.0001$ indicate that the variables, X_1, X_3, X_4, X_1X_2, X_1X_4, X_2X_3, X_1^2, X_2^2, X_3^2, X_4^2 were significant-in model terms. Researchers has proved that if Coefficient of determination (R^2) value lies between 0.90 and 1.00, in regression equation then the fitted model is considered as highly correlated [41,42]. In this study, R^2 of the model was 0.9897 which indicates that, 98.97 % of the experimental and predicted data can be explained by the model. In addition, the value of adjusted determination coefficient Adj R^2 = 0.9794 was also found acceptable, confirms that the model was significant. The value of coefficient of variation (CV = 2.34 %) explained that there is superiority in the experimental data and the model i.e. lower value of CV shows high degree of precision. From the coefficient factors of Table 4, it can be seen that the interaction of pH and agitation-speed has optimistic effect, like vice in agitation-speed and atrazine-concentration. The quadratic terms of pH, temperature, atrazine-concentration, and agitation-speed as well as interactions of pH with temperature, pH with agitation-speed, temperature with agitation-speed and temperature with Atrazine-concentration, have negative effect on atrazine degradation.

3.4. Graphical analysis on atrazine degradation

The relationships between the four variables were resolved with the help of three dimensional response surface plots. The graphical representation of response surface illustrated the effects of two variables on atrazine degradation, by keeping the other variable at fixed level. The 3D-plots and contour plots are displayed in Fig. 3**(A)**–**(F)**. Where, optimization parameters are represented as: A = pH, B = temperature, C = agitation-speed and D = atrazine-concentration. For the optimization of the degradation process, pH is one of the, most important parameter. It was clear from Fig. 3**(A)**, that with increase in pH up to 7.05, the degradation of atrazine increased and then after degradation decreased. Fig. 3**(A)**, **(D)** and **(E)** depicted that atrazine degradation enhanced as the temperature increases uptos 30.4 °C and then the atrazine degradation decreased. It was also represented in Fig. 3**(C)**, **(E)** and **(F)** that as the agitation-speed increases up to 145.7 rpm, atrazine degradation increased and after then decreased. Fig. 3**(B)**, **(D)** and **(F)** showed that with increase in atrazine-concentration the degradation increased upto 200.9 ppm, after that atrazine degradation decreased.

4. Discussion

In Present study, a remarkably successful strain from Bacillus species was chosen for atrazine degradation and optimization of factors through RSM. All results described that a pH (7.05), temperature (30.4 °C), agitation-speed (145.7 rpm) and atrazine-concentration (200.9 ppm), were proved to be the best conditions to obtain maximum atrazine degradation using the bacterial isolate. The model was validated and confirmed by the predicted and the experimental values with their optimal points and appropriately explained the effects of the chosen variables on the atrazine degradation by isolated bacterium. All results of this study shown similarity with other studies in which species like *Rhizobium rhodococcus* (72 %), mixed microbial consortium (50 %), *Pseudomonas aeruginosa* (80 %), *Pseudomonas alcaligenes* (>80 %), *Klebsiella sp. and Comamonas sp.* (83.3 %) were used for degradation [12,13,31,43,44]. Therefore, the bacterial culture (*Bacillus badius* ABP6 strain) isolated from contaminated soil proved to be useful in the development of improved process of atrazine degradation.

Bioremediation methods make an effort to improve the naturally happening biodegradation processes, by optimizing the growth favorable conditions and also enhanced the activity of particular degraders [45]. The size, metabolic-activity and composition-of microorganisms have been recommended as determining factors for the biodegradation practicability [46]. Environmental factors like suitable microorganisms for biodegradation of a specified-contaminants plays important role to study

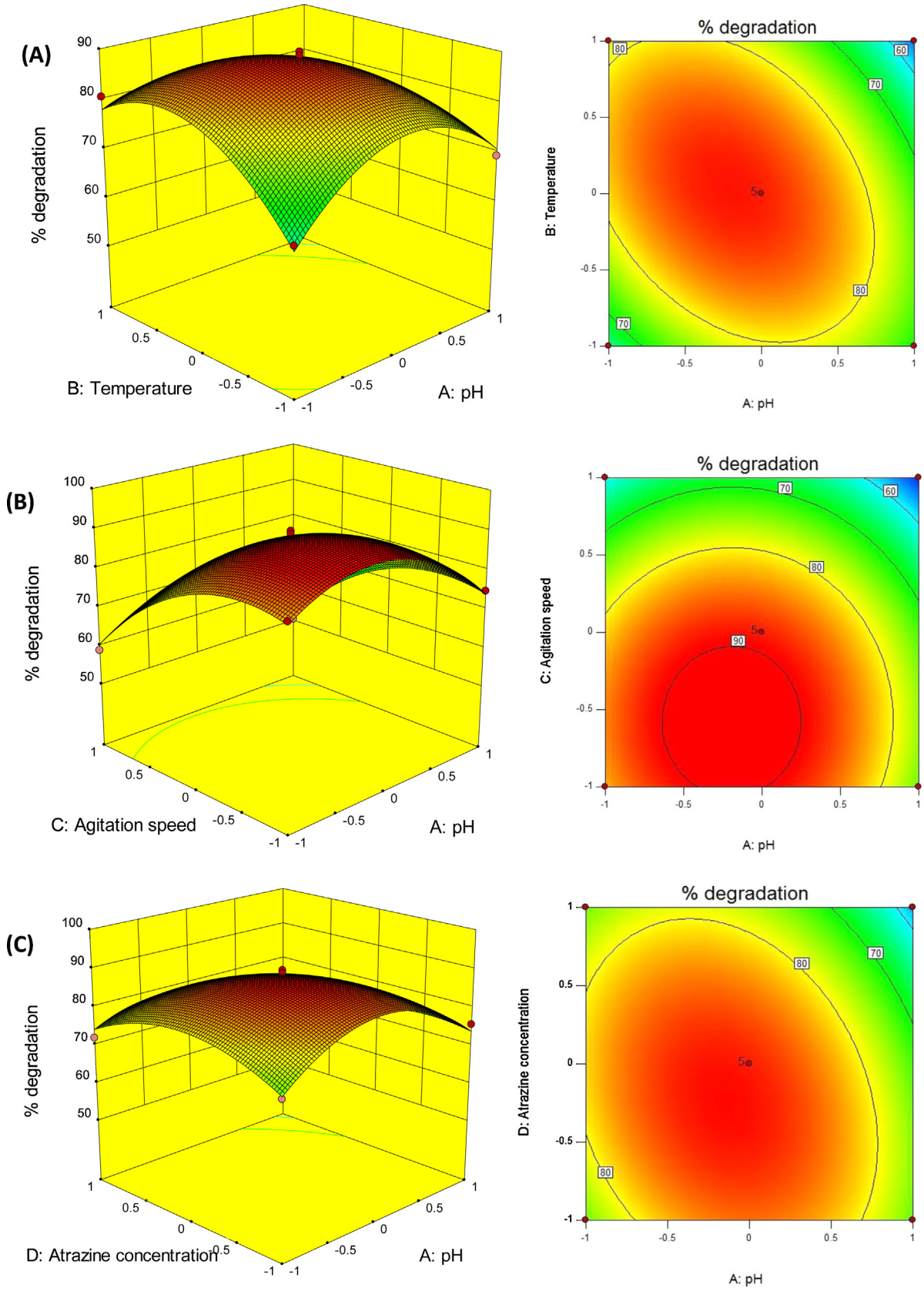

Fig. 3. The 3D plots and Contour plots showing the effect of **(A).** pH and Temperature **(B).** pH and Atrazine-concetration **(C).** pH and Agitation-speed **(D).** Temperature and Atrazine-concentration **(E).** Temperature and Agitation-speed **(F).** Effect of Agitation-speed and Atrazine-concentration on atrazine-degradation.

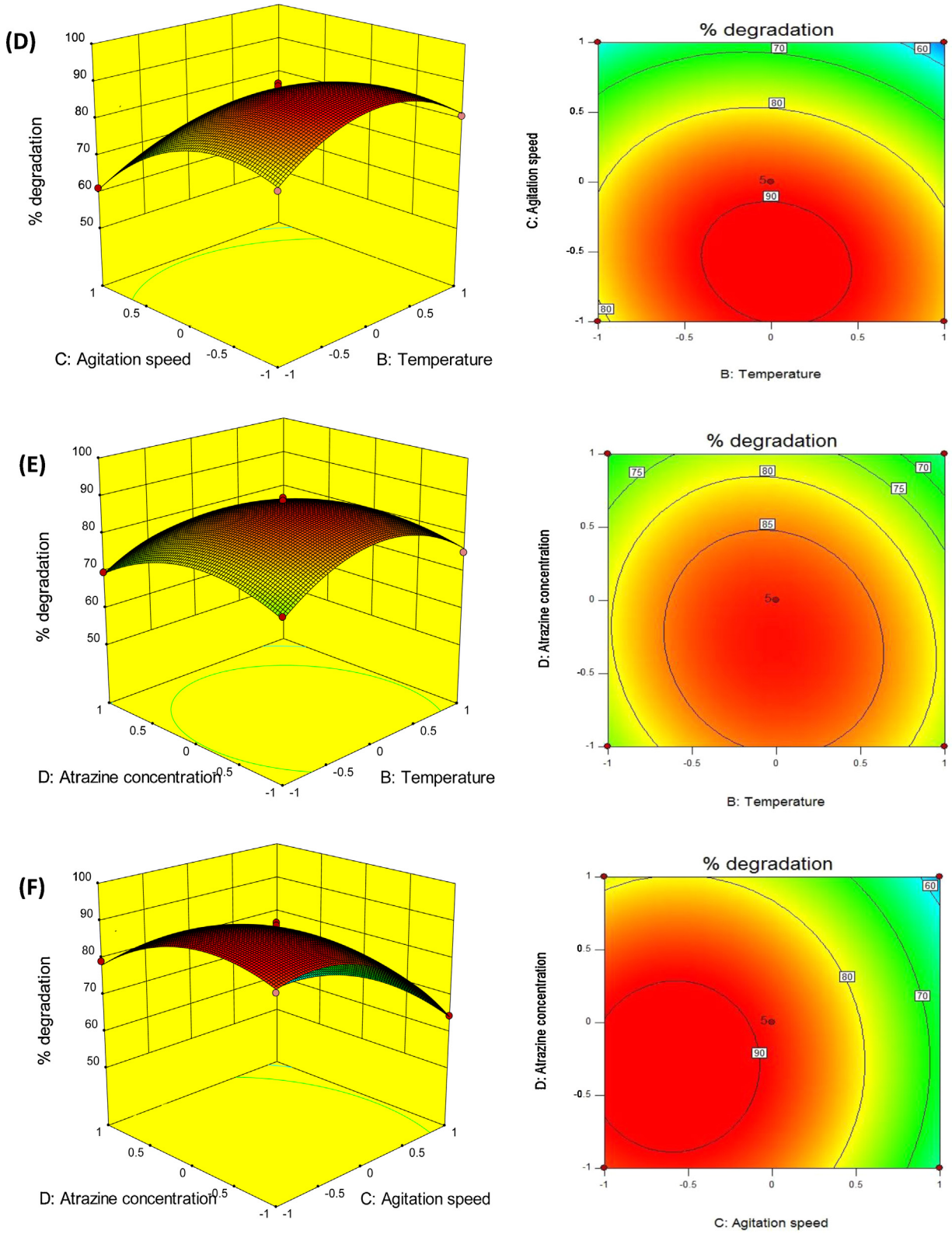

Fig. 3. (Continued)

the optimization conditions for particular response. In microorganisms pH tolerance is quite important for atrazine degradation. Most degrading, bacteria could degrade persistent aromatic compounds such as pesticides at pH range of 6.0 and 8.0, such as *Pseudomonas* [47], *Bacillus* sp.+*Pseudomonas* sp. [48], *Bacillus* sp. [49]. Temperature has also been reported, as one of the most important factor to degrade hazardous chemical compounds [50]. The highest activity and growth of microorganisms with high

pesticide degradation rate was reported at mesophillic temperature range from 30 to 35 °C [48,51]. In our study Atrazine degradation at higher temperature was considerably suppressed because at very high temperature the microorganisms loss their cell viability [49,52]. It was reported that the degradation rate decreases with corresponding increase in agitation-speed [30,53,54]. In our study as the agitation-speed increases the atrazine degradation also increased and after then decreased. That was due to the contact between the microorganisms and the atrazine, which enhanced the degradation of atrazine. Supplementary increase in agitation-speed leads to decrease in atrazine degradation. That was due to the reason that at high speeds, interruption of cells occurs which leads to poor degradation [49]. It was also reported that the increase in atrazine-concentration decreases the degradation efficiency [30]. At higher concentration of atrazine, better degradation results were obtained in our study, because this bacterial strain can capable to survive upto higher-concentration of atrazine [49]. Therefore, this study focuses on the advance optimization-studies of such parameters for atrazine degradation. RSM is less time consuming method as compared to the conventional "single-factor at a time experiment" method of optimization. The interaction and simultaneous effect of various parameters, such as pH, temperature, agitation-speed and atrazine-concentration on percentage-degradation was examined by RSM. Moreover, RSM explained the effect of different factors in a more pronounced way and describes the interactive-effect of different variables on atrazine degradation.

Other researchers used mathematical-quadratic-model and ANOVA for data testing [55]. In the present study, a statistical model based on RSM was applied to optimize the biodegradation conditions of atrazine by isolated *Bacillus badius* strain ABP6. The model was proved as accurate as well as reliable within the limits of selected factors. Box-Behnken design, as used commonly in industrial application because of its economical design, requires only three levels of each factor [56]. Some researchers have also used RSM based on the Central composite and Box-Behnken design to optimize the degradation condition of pyrethroid insecticides at different temperatures, pH and inoculum size, they recognized RSM as a convenient and efficient system [57]. The overall results of the present study shown similarity with the work of other researchers [28–32,58–61] related to optimization using RSM.

5. Conclusion

The Biodegradation study of atrazine was carried out with the bacterium *Bacillus badius* ABP6 strain isolated from contaminated field soil. The RSM was used with isolated bacterium strain for the verification, and optimization, of process parameters, namely pH, temperature, agitation-speed and atrazine-concentration for degradation of atrazine. This statistical analysis technique confirmed as useful and powerful tool, in standardizing optimum degradation conditions. This is the first research which describes the application of statistical designing tool for the degradation of atrazine by *Bacillus badius* ABP6 strain culture and Design expert software (new version 10.0.1). Under the best suitable conditions, the obtained experimental value of percentage degradation was about 89.7 %, which was very close, with the predicted value i.e. 88.6 % as observed from the application of statistical design. From the results, it can be concluded that RSM applied here as optimization tool can be effectively used elsewhere as well, to maximize the degradation process by optimizing degradation parameters.

Author statement

Authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, authors certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Biotechnology Reports Journal.

Author's contributions statement

In this article Miss. Hina Khatoon performed the experiments, generated scientific data in the constant supervision of Dr. J.P.N.Rai. Subsequently Miss. Hina Khatoon wrote the main manuscript text, prepared Figs. 1–3 and Tables 1–4 while Dr. J.P.N.Rai checked and reviewed the whole manuscript critically in order to avoid grammatical mistakes and insure language fluency.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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