



Design and simulation of diatom-based microcantilever immunobiosensor for the early detection of Karnal bunt

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Abstract

Fungal pathogen, *Tilletia indica*, the cause of Karnal bunt disease in wheat, is severely affecting the yield and grain quality, worldwide. Thus, strict quarantine regulations by most wheat growing countries have to be followed, leading to trade barriers for wheat export. The conventional methods being used for pathogen detection at symptomatic stage requires the germination of *Tilletia* spores for the processing of samples. Thus, it is time-consuming and expensive. This study proposes a simulated microcantilever-based piezoelectric biosensor for the early detection of *T. indica*. Four different materials, SiO₂, SiC, Si₃N₄, and Poly Si, were used for the microcantilever design. Microcantilever was coated with siliceous frustules of diatom that provides high surface area and enhanced sensitivity for specific antibody against the antigen, *T. indica*. Ansys software was used for the simulation analysis. Simulation results showed that microcantilever beam of SiO₂ length of 150 µm, width of 30 µm and thickness 1 µm enhanced the sensitivity by two times against the antibody in comparison to normal microcantilever beam. The results concluded that SiO₂ with coated diatom is the best material for the microcantilever fabrication, thus, providing an excellent protocol for fabrication of microcantilever-based biosensor which is both cost- and time effective.

Keywords Karnal bunt · *Tilletia indica* · Diatom frustules · Biosensor · Microcantilever · Simulation

Introduction

Tilletia indica is a fungal pathogen causing the disease Karnal bunt in wheat seeds. This problem is distributed in mainly northwest India to central region where winter and high humidity dominate (Singh et al. 1985). This fungus is also found in the germplasm of wheat exported to other countries. Infection occurs in the whole ears of wheat and leads to crop damage which makes this disease more difficult to identify and leads to a major infection of wheat crops before it is fully realized. It is very difficult to eradicate teliospores of this fungus from soil as it remains viable for years in soil. Due to this there are high yield losses in susceptible seeds and quality is also affected.

Spores of *T. indica* are called teliospores. Its shape varies from globuse to subglobuse with dark red to brown colour having attached mycelia fragments of 24–47 µm diameter and 1.7–4 µm height. Symptoms of Karnal bunt depend upon the climatic conditions as it prevails in warm and cool humid conditions. Due to this disease the length of ears and spikelet numbers decrease gradually. It develops dusty and brown to black spores. During the initial stage only black point structure below the embryo is apparent. In severe form, several tissues and endosperms are filled with spores. The glumes open apart and expose the infected grains and then both glumes as well as grains fall on the ground.

This disease became the target of quarantine regulations in most of the wheat-growing countries and this leads to trade barriers for the wheat exporters and is now called as biological weapons (Schaad et al. 1999). The detection of this disease can be done only when crops are severely affected and showing the symptoms and not in the early stage of infection. Current laboratory-based methods such as visual microscopic examination and immunological and DNA-based assays for the detection of these spores are expensive and time-consuming (Micales et al. 1986; Chesmore et al. 2003; Kumar et al. 2008).

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There is an urgent requirement of fast, specific and economical immunobiosensors which can detect selective target antigen of *T. indica* through the interaction with highly specific antibody. Among all biosensors, microcantilever array biosensors are based on the technique of optical detection which measures various surface stress-induced beam deflections. When target analyte/molecules come in contact with this microcantilever and get attached to the functionalized surface of the beam, the altered surface stress will cause deflections. This explains that higher deflections exhibit higher sensitivity in microcantilever biosensor (Chhabra and Sharma 2012). However, major challenge for the development of microcantilever-based biosensor is to achieve the sensitivity in smaller detection range for on-site analysis. This sensitivity depends on the ability of device for conversion of biochemical reactions into microlevel mechanical motions. The range of these deflections is from tens to hundreds of nanometers. The current paper proposes the simulation of new design of microcantilever immunobiosensor with one fixed end and other free end that will harbour the detection platform. This is more sensitive than other conventional methods used (Sodano et al. 2004). The proposed work utilizes the three-dimensional (3D) characteristics of siliceous diatomic frustules, for enhanced sensitivity as compared to current 2D biosensor. Diatoms have been used in several fields including biophotonic and biosensing devices, as they are nontoxic, highly stable and reusable (Leonardo et al. 2015).

Material and methods

Design parameters

The geometry T-shaped cantilever beam is anchored at one end of the microcantilever body while the other end is free. The length of the beam is greater as compared to its width and thickness (Fig. 1).

Different dimensions of cantilever beam is hypothesized to find the best design of microcantilever with maximum deflection.

Design constraints

The physical representation of microcantilever biosensor is rectangular in shape and design parameters of this is mainly based on three formulas (Arora et al. 2012).

(a) Stone's formula: This is used for the calculation and analysis of the deflection of microcantilever. This is given by the following equation:

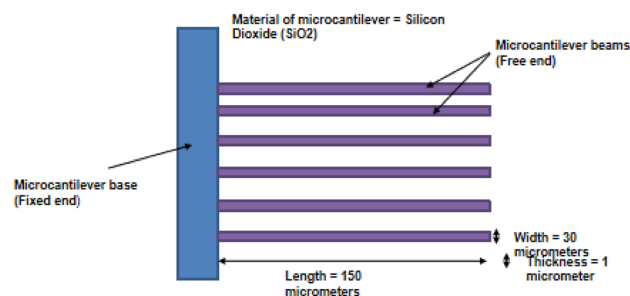


Fig. 1 Proposed multicomb microcantilever design with SiO₂

$$\delta = \frac{3\sigma(1-\nu)L^2}{Et^2},$$

where δ is beam deflection, ν is Poisson's ratio, σ is applied stress, E is Young's modulus; L is beam length and t is cantilever thickness.

Deflection of microcantilever is dependent on two factors, i.e. dimension of structure and the type of materials used for the construction of its structure. It also depends on the stiffness of the materials used for the constructions.

(b) Spring constant formula (K): This formula relates the deflection which is observed as follows:

$$K = \frac{F}{\delta} = \frac{Ewt^3}{4L^3k}.$$

Here, F is the applied force and δ is applied stress, w is width, t is thickness of the cantilever beam E is young modulus, L is total length and k is rotational stiffness.

(c) Hooke's law: This explains the relation between the applied force and the displacement. Therefore, the extra mass exerts additional force on cantilever beam which increases the displacement of the beam. This law is mathematically represented by $F = -KX$. The negative sign represents the exerted restoring force.

These equations help us to understand the mechanism and behaviour of MEMS based microcantilever.

Experimental analysis

For fabrication or physical designing of microcantilever sensor, Ansys software was used for the simulation and analysis of the materials. Various steps were performed for the simulation which includes identification of the materials, defining the geometry of materials, meshing, applying formula and analysis of results. The structure of microcantilever depends upon the above formulas used and the two positions of the platform. Static position defines stationary and dynamic status defines frequency analysis. A microcantilever is simplest mechanical structure which can be used as sensor, where its

Table 1 Different dimensions used with each selected material to check the simulation

Dimensions	A	B	C
Length (μm)	150	300	500
Width (μm)	30	60	100
Thickness (μm)	1	2	4

Table 2 Material properties of selected materials

S. no.	Material properties	Materials			
		SiO ₂	SiC	Si ₃ N ₄	Poly Si
1	Density (kg/m^3)	2200	3216	3100	2320
2	Young's modulus (Y) (GPa)	70	748	250	169
3	Possion's ratio	0.17	0.45	0.23	0.22

upper surface is coated with a specific sensing layer. 3-D rectangular cantilever was designed by adding different constraints.

Selected materials

For the simulation of microcantilever design four different materials were considered SiO₂, SiC, Si₃N₄ and Poly Si.

Design of microcantilever

The design of rectangular microcantilever was simulated with three different dimensions (see Table 1).

The material properties were listed in the Table 2 for optimization.

The lower surface of the rectangular beam was coated with three-dimensional network of frustules of diatom as they provide larger surface area and enhance the sensitivity. This will enhance the signal and fabricate highly sensitive biosensors with high signal to noise ratio. Another study showed the DNA-based nanosensor by using NH₂ labelled standard probe against infectious gene of *Salmonella enterica* in milk samples (Saini et al. 2019). Large-scale fabrication of 3D nanostructures on small chip is an essential requirement for the commercialization (Gopinath et al. 2014). The upper layer of the diatom was immobilized with the antibody against *T. indica* (Fig. 2).

The initial displacement with a force of 3.98×10^{-21} Pa was observed for the beam coated with diatoms and antibody. Theoretically, for an immunocomplex to show displacement $\times$ number of antigen molecules has to bind with $2 \times$ molecules of antibody which is immobilized on a diatom coated beam (Table 3).

Thus, we could predict that the target antigen was getting attached to the antibody. For optimization of microcantilever the net displacements for the initial conditions and those after the reaction were simulated.

Fig. 2 Schematic representation of 3D siliceous microcantilever-based biosensor **a** resting state and **b** sensing state (patent filed by Mishra et al. 2019)

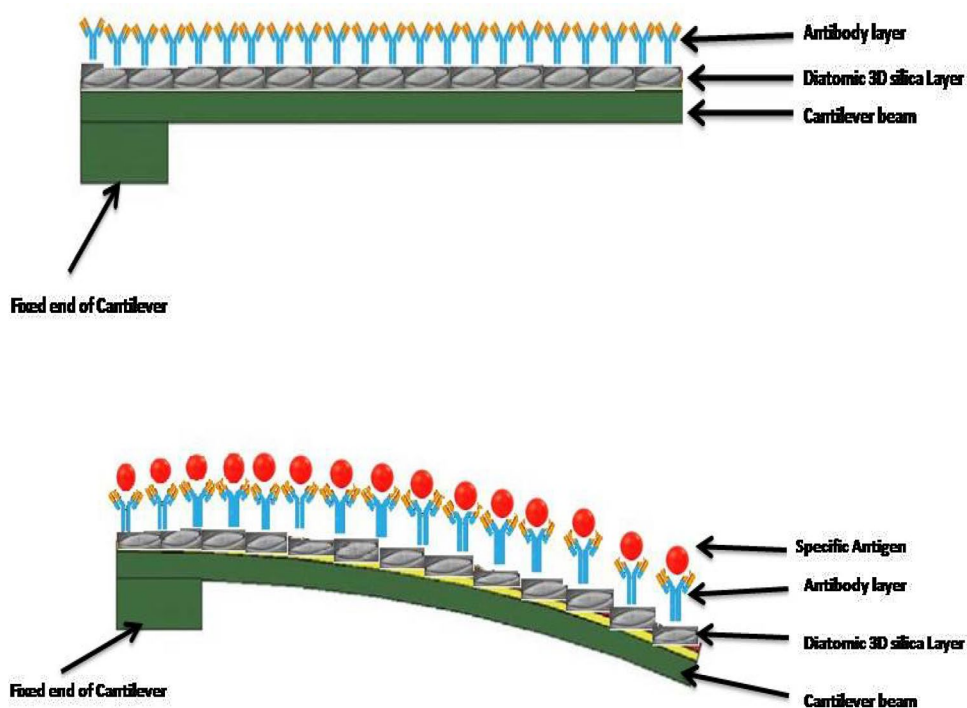


Table 3 Total force calculated of different molecules coated on microcantilever beam

S. no.	Coatings on beam	Weight in KDa	No. of molecules attached	Total molecular weight KDa
1	Diatom molecules	200	5	1000
2	Antibody molecules	28	20	560
3	Antigen molecules	34	10	340

Results and discussion

The simulation of microcantilever biosensor is an essential step before its fabrication. In this study, the microcantilever beam was fixed at one end and the other end was free. The free end was coated with the frustules of diatom *Navicula* sp. Further, microcantilever was coated with the specific antibody against *T.indica*. In order to optimize the type of material for microcantilever beam, four different materials SiO_2 , SiC, Si_3N_4 and poly Si were tested with three different dimensions, i.e. A, B and C as shown in Table 1.

Ansys software was used for simulation. This software is mainly used to optimize the design of various products and semiconductors, to help create simulations that check the product's durability, fluid movements, temperature distribution and electromagnetic properties.

After the addition of specific target antigen *T. indica*, the best deflection was observed in case of SiO_2 material with dimension A. SiO_2 was seen to be more sensitive

than the other selected materials. The simulations were calculated with total weight of diatom *Navicula* sp., antibody against *T.indica* (initial reaction) and after addition of antigen (spores of *T.indica*) the combined weight of diatom-antibody-antigen was observed (after reaction) for each material taken and showed in Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14.

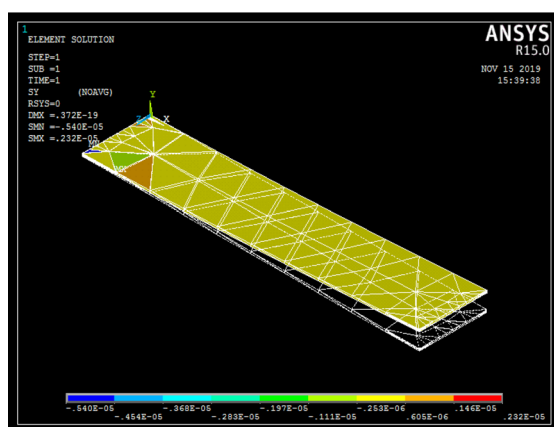
The simulation results provides us an insight into displacement and stress values (see Table 4) for different materials used for the experimental design.

In case of dimension A, the silicon oxide showed significant results in different attributes like maximum displacement after addition of antigen, i.e. from 0.372 e^{-19} to 0.538 e^{-19} , minimum stress level from -0.540 e^{-5} to -0.336 e^{-5} and maximum stress from 0.232 e^{-5} to 0.781 e^{-5} compared to other materials (see Tables 4, 5, 6).

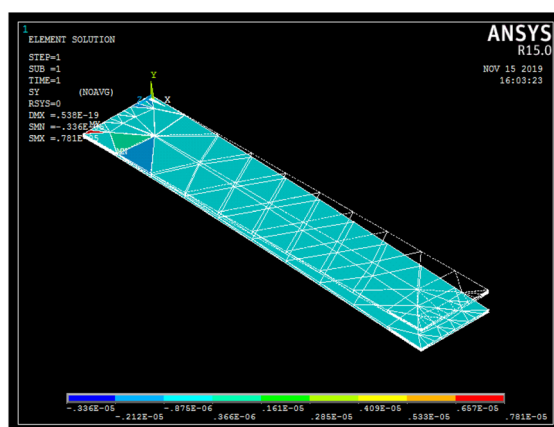
In case of dimension B, the silicon oxide showed significant results in different attributes like maximum displacement after addition of antigen, i.e. from 0.816 e^{-19} to 0.269 e^{-19} , minimum stress level from -0.580 e^{-5} to

Simulation with SiO_2

Dimension A



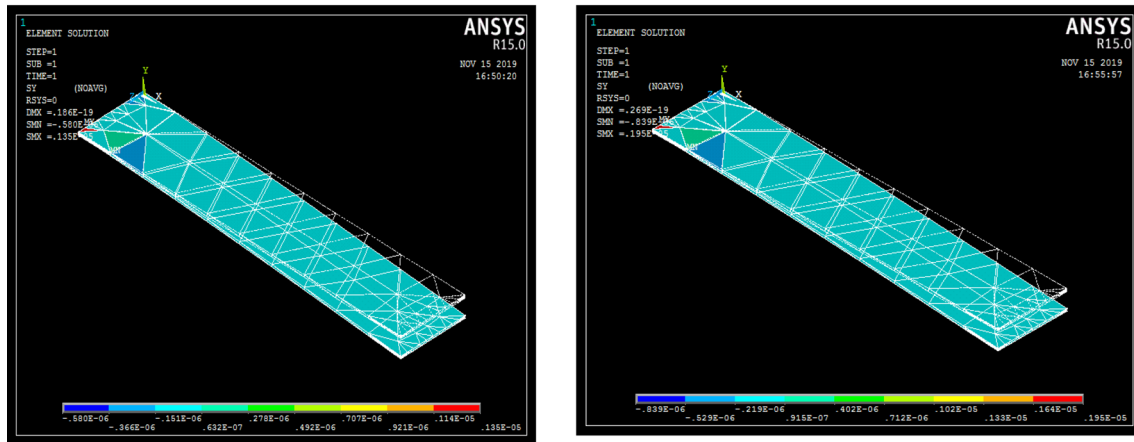
A Diatom +Antibody



B Diatom+Antibody + Antigen

Fig. 3 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of SiO_2 material with dimension A

Dimension B

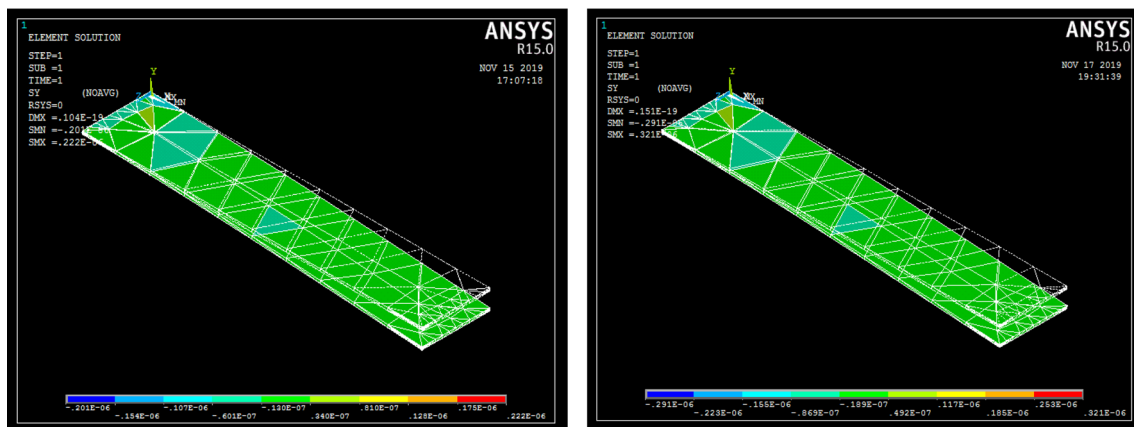


A Diatom +Antibody

B Diatom+Antibody + Antigen

Fig. 4 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of SiO₂ material with dimension B

Dimension C



A Diatom +Antibody

B Diatom+Antibody + Antigen

Fig. 5 Microcantilever simulation results of initial and after addition of antigen on antibody coated beam of SiO₂ material with dimension C

– 0.839×10^{-6} and maximum stress from 0.135×10^{-5} to 0.195×10^{-5} compared to other materials (see Tables 4, 5, 6).

In case of dimension C, the silicon oxide showed significant results in maximum displacement after addition of antigen, i.e. from 0.104×10^{-19} to 0.151×10^{-19} .

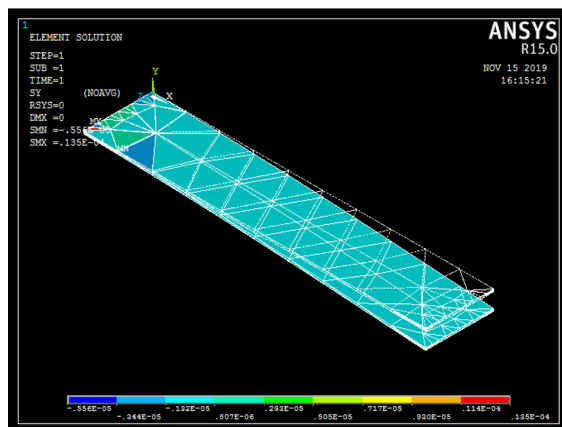
In case of stress values, the minimum stress level was best in SiC from -0.409×10^{-6} to -0.592×10^{-6} and maximum stress seen in SiC from 0.259×10^{-6} to 0.375×10^{-6} compared to other materials (see Tables 4, 5, 6).

In conclusion, the graphical representation of all selected materials with standard deviations is shown with different dimensions (Figs. 15, 16, 17).

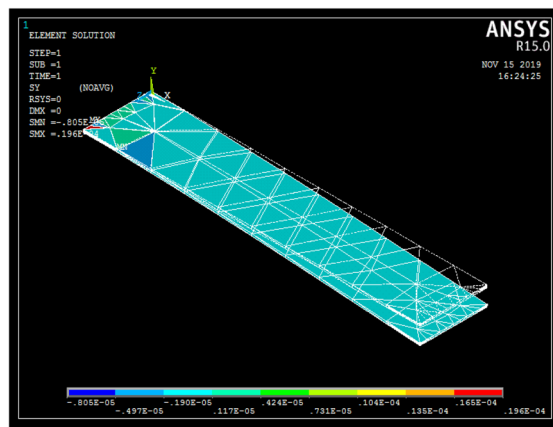
From these following simulations it is suggested that the force applied to the microcantilever beam will be kept same and we can compare and get the best geometrical dimensions for implementing different applications.

For our study we have chosen SiO₂ material that can bend easily with low applied external pressure which gives the

Dimension A



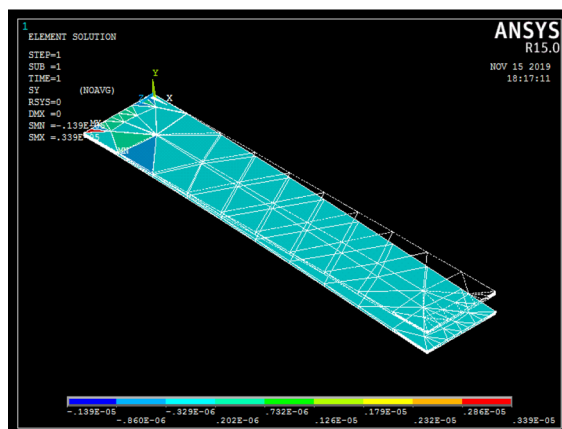
A Diatom +Antibody



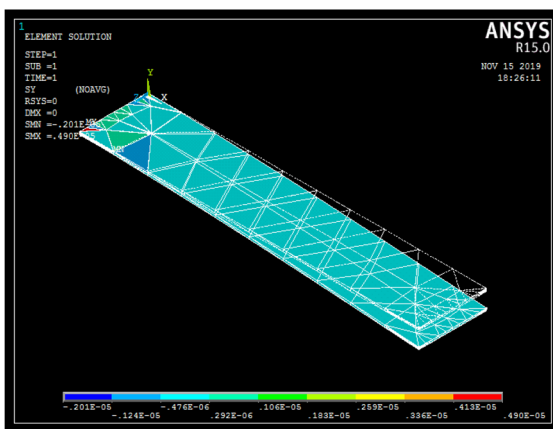
B Diatom+Antibody + Antigen

Fig. 6 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of SiC material with dimension A

Dimension B



A Diatom +Antibody



B Diatom+Antibody + Antigen

Fig. 7 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of SiC material with dimension B

enhanced sensitivity for antigen detection due to the hierarchically distributed three-dimensional layer of diatom frustules on the beam (De Stefano and De Stefano 2005). The special feature of this microcantilever biosensor is its enhanced sensitivity due to using the layer of diatom frustules on the beam.

The sensing application of diatomic frustules have also been reported by many researchers (Sarika et al. 2015; Kong et al. 2016, 2017, 2018; Selvaraj et al. 2018; Rea et al. 2019; Sivashanmugan et al. 2019a, b; Gannavarapu et al. 2019). In our work, the simulation showed the bending of the beam at its tip with the optimized length of 150 μm for width 10 μm and thickness 1 μm . For this dimension of beam the displacement 0.538 e^{-19} was observed. The mechanism

behind the bending of microcantilever is due to interaction of biosensing elements on the surface which further optically sensed by a transducer. Bumbu et al. (2007) have also shown the static mode technique for the study of nature of methyl methacrylate brushes, polymerized form of silicon surface using a “grafting” approach. The microcantilever sensors are proven to be highly sensitive platforms for the detection of different molecular interactions which is label-free, and less time-consuming.

During the fabrication design, as the mass loading increases the resonance frequency of a microcantilever shifts and deflection occurs (Boisen and Thundat 2019). This bending of microcantilever beam was found as differential surface stress changes during adsorption process. Hansen

Dimension C

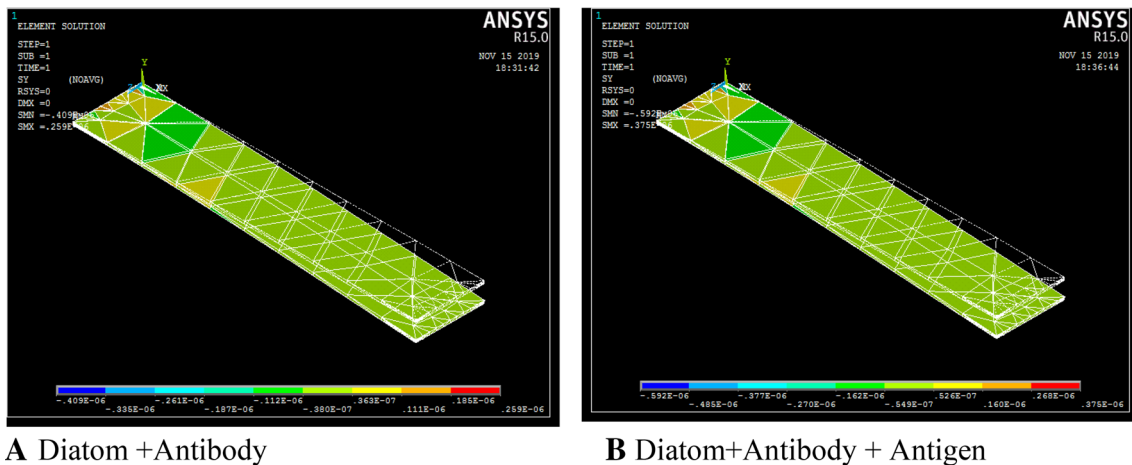


Fig. 8 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of SiC material with dimension C

Dimension A

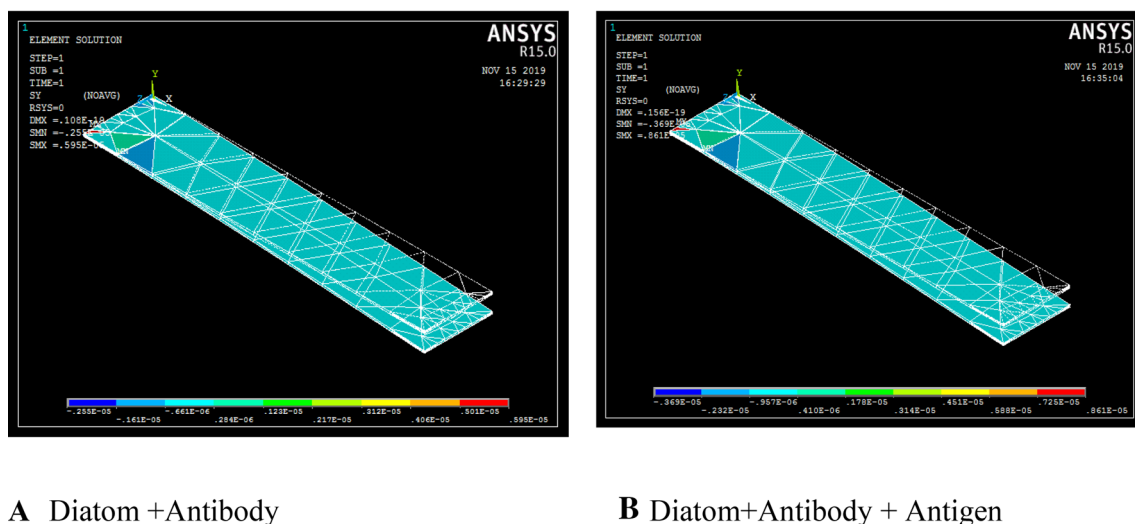


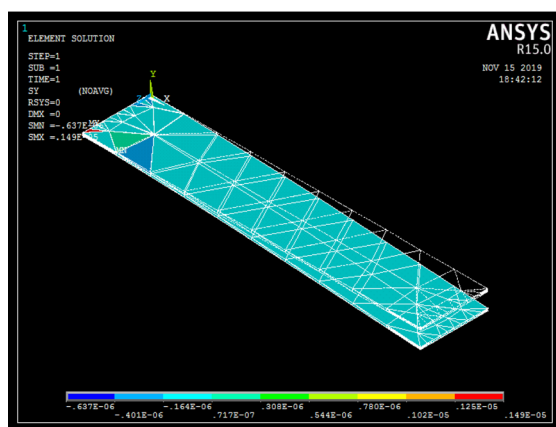
Fig. 9 Microcantilever simulation results of initial and after addition of antigen on antibody coated beam of Si₃N₄ material with dimension A

and Thundat reviewed the use of microcantilever technology for highly sensitive biosensor applications based on microcantilevers (Hansen and Thundat 2005). The microcantilever biosensor is the right detection tool for the early detection and ensuring the good quality of plant (Oluwaseun et al. 2018). However, the methods of antibody–antigen interactions will be appropriate for quality testing of suspicious plants for surveillance management. Patkar et al. (2018) demonstrated the use of microcantilever platform in both mode of operations, i.e. static and dynamic mode for the detection of pathogen *Ralstonia solanacearum* antigens by using ultrasensitive Mecwins® SCALA biosensor platform

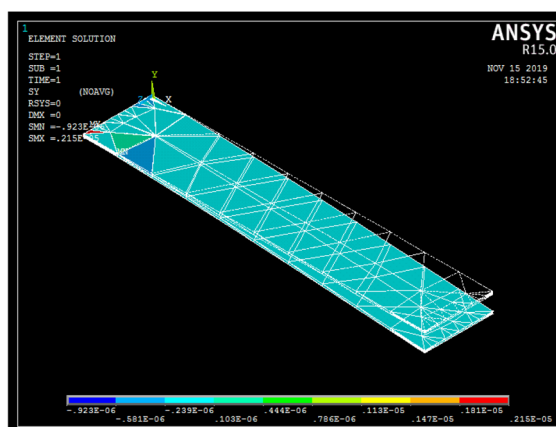
in liquid medium. In another study, Zopf et al. (2019) demonstrated that the multiplexed detection and identification of various fungal pathogen DNA sequences which was subsequently immobilized on one sensor with many arrays and utilized the spotted gold nanoparticle as sensing platform. Khashayar et al. (2017) also developed the highly sensitive electrochemical biosensor which was based on AuNP gold electrodes for determination of serum levels.

Therefore, this diagnostic tool is useful for screening of large amounts of plant diseases which could be effective during on-site evaluation and pre and post testing probability of disease risk. The innovative field-based devices, required for

Dimension B



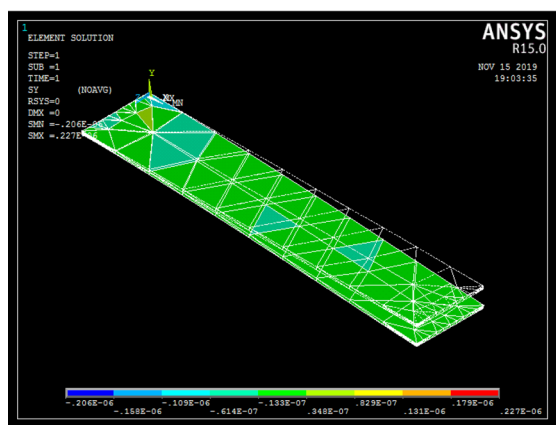
A Diatom +Antibody



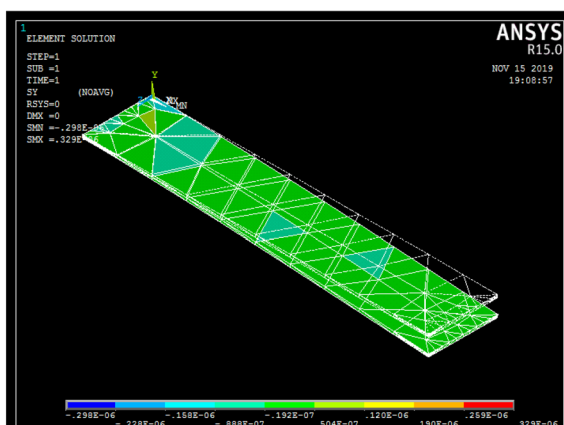
B Diatom+Antibody + Antigen

Fig. 10 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of Si_3N_4 material with dimension B

Dimension C



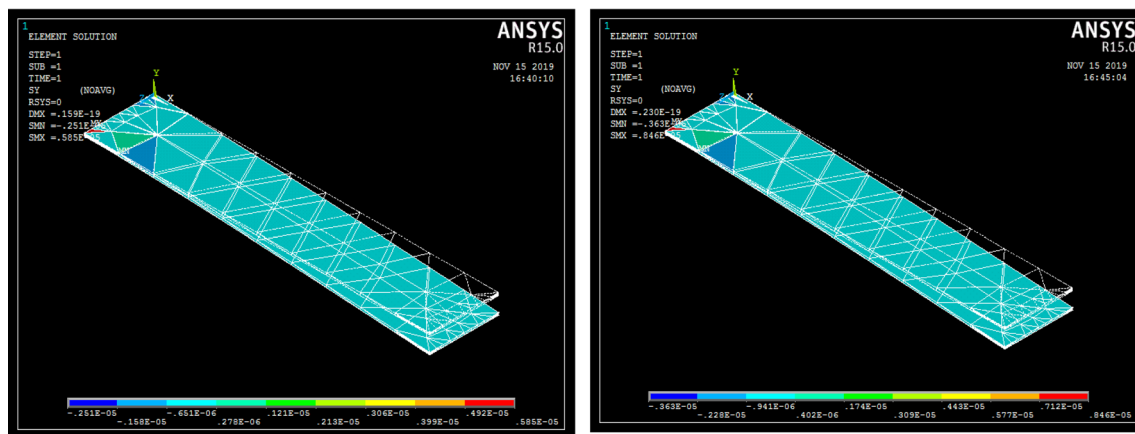
A Diatom +Antibody



B Diatom+ Antibody + Antigen

Fig. 11 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of Si_3N_4 material with dimension C

Dimension A

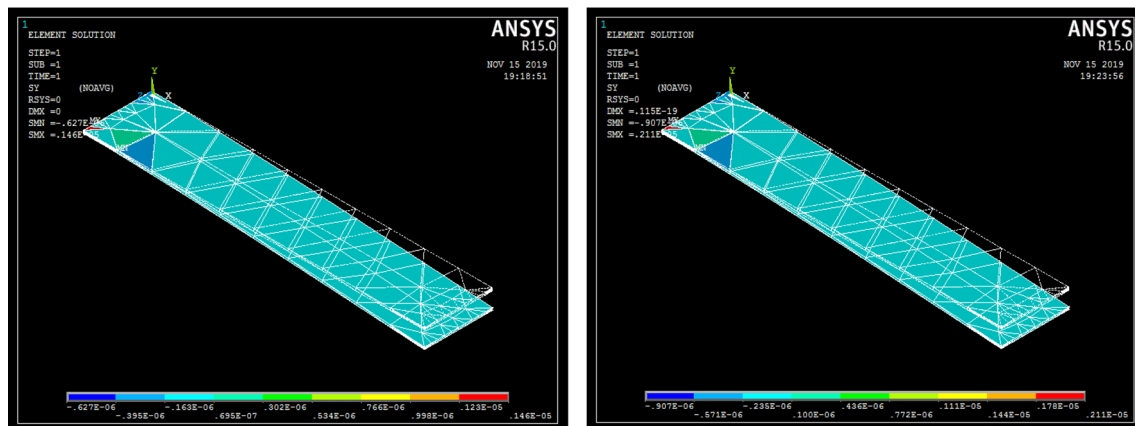


A Diatom +Antibody

B Diatom+Antibody + Antigen

Fig. 12 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of Poly Si material with dimension A

Dimension B



A Diatom +Antibody

B Diatom+Antibody + Antigen

Fig. 13 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of Poly Si material with dimension B

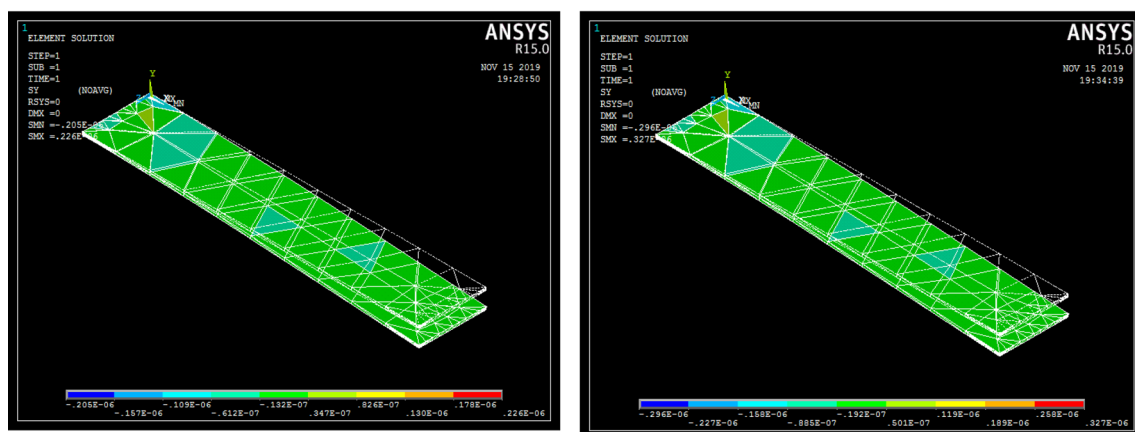
novel approaches which could limit the spread of plant diseases across national and international borders (Khater 2017; Boris et al. 2020).

Conclusion

World economy is hugely decreasing as the majority of crops are suffering from plant pathogens especially fungus, *T. indica*. Microcantilever biosensor based on antibody

antigen reaction immobilized on diatom frustules will be a novel idea for the early detection of antigens as it is label-free and real-time estimation. From the above simulation results, we can conclude that the maximum displacement and stress of microcantilever beam were significantly increasing when it was simulated with SiO_2 material with dimension A. The deflection of beam lies in the range of nanometers which showed its sensitivity in low availability of fungal pathogens. The ultimate goal of this work was to finalize the best material and design parameters

Dimension C



A Diatom +Antibody

B Diatom+Antibody + Antigen

Fig. 14 Microcantilever simulation results of initial and after addition of antigen on antibody-coated beam of Poly Si material with dimension C

Table 4 Displacement and Stress level of microcantilever observed in different materials with different dimensions (simulation with dimension A)

Properties analysed	SiO ₂		SiC		Si ₃ N ₄		Poly Si	
	Ab	Aa	Ab	Aa	Ab	Aa	Ab	Aa
DMX (maximum displacement)	0.372 e ⁻¹⁹	0.538 e ⁻¹⁹	0	0	0.108 e ⁻¹⁹	0.156 e	0.159 e ⁻¹⁹	0.230 e ⁻¹⁹
SMN (minimum stress)	- 0.540 e ⁻⁵	- 0.336 e ⁻⁵	- 0.556 e ⁻⁴	- 0.805 e ⁻⁰⁶	- 0.255 e ⁻⁰⁵	- 0.396 e ⁻⁰⁶	- 0.251e ⁻⁰⁶	- 0.363 e ⁻⁰⁶
SMX (maximum stress)	0.232 e ⁻⁵	0.781 e ⁻⁵	0.315 e ⁻⁰⁴	0.196 e ⁻⁰⁴	0.595 e ⁻⁰⁵	0.861 e ⁻⁰⁵	0.585 e ⁻⁰⁵	0.846 e ⁻⁰⁵

Aa dimension A before adding antigen, Ab dimension A after adding antigen

Table 5 Displacement and stress level of microcantilever observed in different materials and with different dimensions (simulation with dimension b)

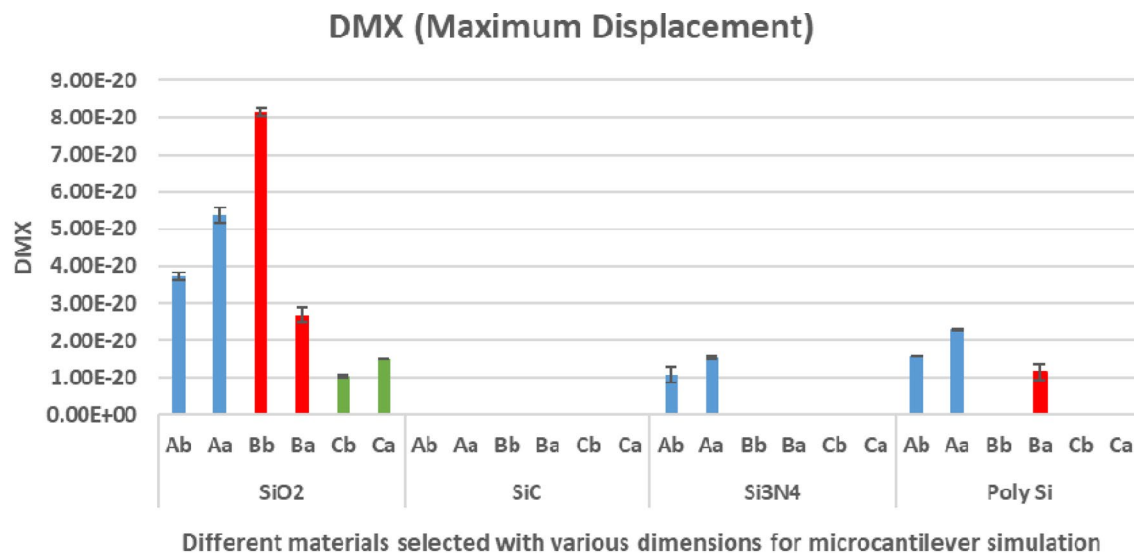
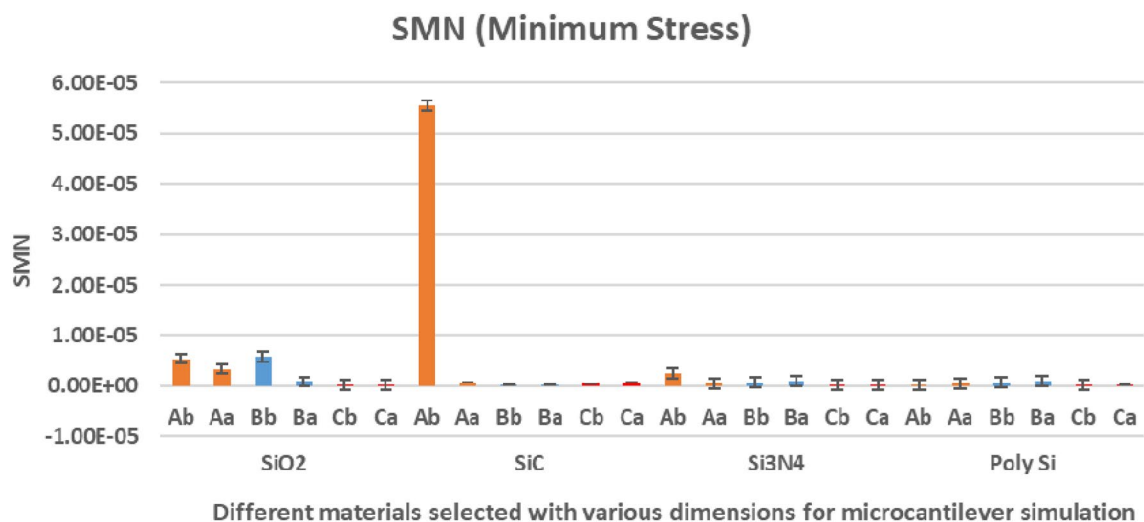
Properties analysed	SiO ₂		SiC		Si ₃ N ₄		Poly Si	
	Bb	Ba	Bb	Ba	Bb	Ba	Bb	Ba
DMX (maximum displacement)	0.816 e ⁻¹⁹	0.269 e ⁻¹⁹	0	0	0	0	0	0.115 e ⁻¹⁹
SMN (minimum stress)	- 0.580 e ⁻⁰⁵	- 0.839 e ⁻⁰⁶	- 0.139 e ⁻⁰⁶	- 0.201 e ⁻⁰⁶	- 0.637 e ⁻⁰⁶	- 0.923 e ⁻⁰⁶	- 0.627 e ⁻⁰⁶	- 0.907 e ⁻⁰⁶
SMX (maximum stress)	0.135 e ⁻⁰⁵	0.195 e ⁻⁰⁵	0.339 e ⁻⁰⁵	0.490 e ⁻⁰⁵	0.149 e ⁻⁰⁵	0.215 e ⁻⁰⁶	0.146 e ⁻⁰⁵	0.211 e ⁻⁰⁵

Ba dimension B before adding antigen, Bb dimension B after adding antigen

Table 6 Displacement and stress level of microcantilever observed in different materials and with different dimensions (simulation with dimension c)

Properties analysed	SiO ₂		SiC		Si ₃ N ₄		Poly Si	
	Cb	Ca	Cb	Ca	Cb	Ca	Cb	Ca
DMX (maximum displacement)	0.104 e ⁻¹⁹	0.151 e ⁻¹⁹	0	0	0	0	0	0
SMN (minimum stress)	- 0.201 e ⁻⁰⁶	- 0.291 e ⁻⁰⁶	- 0.409 e ⁻⁰⁶	- 0.592 e ⁻⁰⁶	- 0.206 e ⁻⁰⁶	- 0.298 e ⁻⁰⁶	- 0.205 e ⁻⁰⁶	- 0.296 e ⁻⁰⁶
SMX (maximum stress)	0.222 e ⁻⁰⁵	0.321 e ⁻⁰⁶	0.259 e ⁻⁰⁶	0.375 e ⁻⁰⁶	0.227 e ⁻⁰⁶	0.329 e ⁻⁰⁶	0.226 e ⁻⁰⁵	0.327 e ⁻⁰⁶

Ca dimension C before adding antigen, Cb dimension C after adding antigen

**Fig. 15** Graphical analysis of microcantilever displacements and stress values with different materials and dimensions**Fig. 16** Graphical analysis of microcantilever displacements and stress values with different materials and dimensions

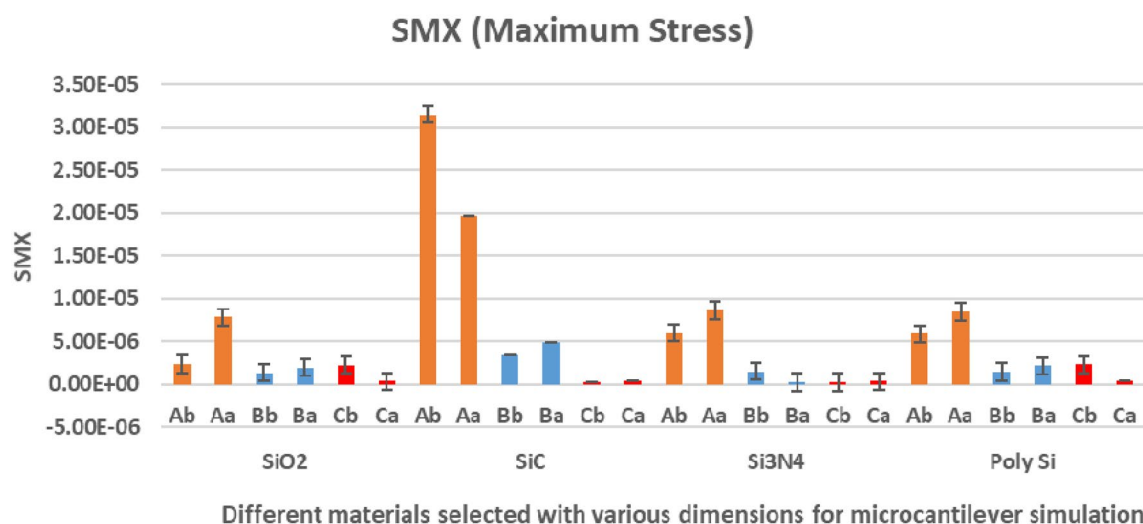


Fig. 17 Graphical analysis of microcantilever displacements and stress values with different materials and dimensions

for the fabrication of microcantilever biosensor making them more sensitive, time saving and accurate for antigen detection in the field/store houses.

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Author contributions MM and SS has conceived and designed the experiments. MM and RS performed the simulations. MM, SS and SSu analyzed the data. MM wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Patent filed Microcantilever-based 3D biosensor made from diatom biogenic silica to detect Karnal bunt pathogen *Tilletia indica*, application no. CBR number: 22790, CBR date: 18-07-2019.

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