Optimization of fermentation parameters for the production of antimicrobial compounds by Fusarium falciforme MNP1 isolated from rhizosphere soil of Epipremnum aureum

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Short Report

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Abstract

In the present study, *Fusarium falciforme* MNP1 (Gen Bank Accession no. PX106850) was isolated from the rhizosphere soil of *Epipremnum aureum* (Money Plant), which showed the remarkable antimicrobial activity against the test microorganisms (*Escherichia coli* MTCC-40, *Pseudomonas aeruginosa* MTCC-647, *Bacillus subtilis* MTCC- 2274, *Staphylococcus aureus* MTCC-6908, *Aspergillus niger* MTCC- 281 and *Candida albicans* MTCC- 183). One Factor at a time (OFAT) and Response Surface Methodology (RSM) were used for the optimization of the fermentation parameters for antimicrobial production. In the OFAT design, fermentation parameters such as pH (4-8), temperature (15-35℃), incubation period (7-15 days), inoculum size (0.25- 1.25 ml) and fermentation vessel size (250- 2000 ml) were considered. Response surface methodology (RSM) based central composite design (CCD) model was performed to evaluate the interactions and the quadratic effect on the antimicrobial metabolites production which inhibit the test microorganisms (responses) in terms of the diameter of zone of growth inhibition (mm). Three variables namely incubation time (days), pH, and temperature (℃) were employed with RSM and investigated with the help of quadratic equations to increase the production of bioactive metabolites. The experimental data to maximize the production of bioactive metabolite was significantly fit by using the second polynomial model. Regression analysis revealed that the model was found significant on the basis R^2 values for all the six responses and values of adjusted R^2 demonstrated the perfect agreement with predicted and experimental values. Methodology (RSM) enhanced the production of bioactive compounds by *Fusarium falciforme* with optimized conditions pH 6, 25℃ temperature and 9 days of incubation period. By using the central composite design, the accurate values of fermentation parameters for the production of antimicrobial compound can

be concluded and further characterization of the antimicrobial metabolites is needed for future prospect.

Keywords: Rhizosphere, fungi, OFAT, RSM, Optimization, Fermentation

Introduction

Presently, the emerging and re-emerging infectious diseases have enormous impact on world which cannot be dramatized and neither underrated. It demands significant attention to find the effective solution to combat the health problems (Ferraz, 2024). Aside from other physiological diseases such as cancers and neurodegenerative disorders, certain infectious diseases like Zika, Ebola, West Nile, influenza, food-borne illness, and global pandemics like HIV, TB, and malaria are having dramatic outcome to the whole world. In fact, more than 20 significantly resistant microorganisms have been pinpointed by the WHO globally, for which new drugs are desperately needed (Aslam *et al.,* 2018)

Antimicrobial resistance (AMR) is the ultimate reason to worsen the situation and some are remarkable factors responsible for the AMR development includes evolving new infectious strains, delays in accurate diagnoses, insufficient knowledge, improper utilization of diagnostic procedures, overpopulation, non- human use, cross-infections, poor health infrastructural facilities (Logan and Weinstein, 2017; Owen and Liard, 2020; Haque *et al*., 2022). Increment in the AMR amongst infectious microbes to almost all class of antibiotics is a notable health crisis all over the world. In the recent past, Vancomycin-resistant *Enterococcus faecium*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and Penicillin-resistant *Streptococcus pneumonia,* and some of the other pathogenic organisms are increasing significantly (Deshmukh *et al*., 2015). More than 1.5 million deaths associated with respiratory infections in the year of 2019 and more than 3.57 million deaths around the world were recorded due to resistance against the existing antibiotics (Murray *et al*., 2022). To combat the escalating AMR, researchers have to investigate the novel scaffolds of the drugs, possessing different mode of action and wide range of antimicrobial activity. Exploring the un-investigated natural habitats to isolate the novel drug scaffolds may enhance the chances of discovery of effective novel bioactive metabolites. Microorganisms are the ray of hope in the middle of these health issues, due to their ability to synthesise various effective compounds to cure the health problems that humanity faces. These bioactive metabolites from the natural resources such as rhizosphere soil, endophytes, marine ecosystems include antibiotics, phytochemicals, toxic substances, algicides or fungicides that exhibits significant antimicrobial activity (Sturza *et al*., 2023). Soil

is one of the diverse sources of bioactive metabolites producing microorganisms, especially, the rhizosphere which is the most active portion with biogeochemical processes influence a host plant and residing microflora. The rhizosphere is the region around a plant root that is inhabited by a unique population of microorganisms (fungi, bacteria, actinomycetes, viruses) influenced by the substances released from plant roots (Luo *et al*., 2024).

Epipremnum aureum (money plant) is one of the most popular ornamental evergreen shrub having heart-shaped leaves in every house, especially, native to South eastern Asia and New Guinea. It is popular to have the capacity to remove the indoor pollutants such as xylene, formaldehyde and benzene (Ali, 2018).

Microorganisms, especially fungi are substantial source of the bioactive metabolites that have potential to combat the several health risks. The genus *Fusarium* is most diverse and highly reported genus comprising more than 70 species which are known to produce the variety of bioactive metabolites (e.g., phenols, flavonoids, alkaloids, saponins, and terpenes) with a wide range of biological properties comprising antioxidant, antidiabetic, antibacterial, antifungal, and cytotoxic activities (Li *et al*., 2020; Shao *et al.*, 2024; Purushothaman *et al*., 2025). Earlier, the *Fusarium* genera were known to cause infectious disease in plants, animals, and humans that make it pathogenic, complex and destructive genus worldwide. *Fusarium* is generally recognized as a diverse genus that typically forms colourless, cotton-like, and hyaline colonies (Balouiri *et al.*, 2016). *Fusarium* represents a large cosmopolitan genus comprising more than 70 species capable of producing a wide array of active metabolites (Summerell and Leslie, 2011). However, various environmental and nutritional factors affect the production of bioactive metabolites. To enhance the bioactive metabolite production during microbial fermentation, the fermentation conditions must be optimized. The best fermentation conditions (i.e., pH, temperature, incubation period, etc.) are optimized for the design of the production medium.

One-Factor-At-a-Time (OFAT) design and Response surface methodology (RSM) experiments have been the most popular method to optimize the fermentation conditions for the production of antimicrobial compound. RSM is an effective mathematical and statistical method for designing the experiments to evaluate variables' effects, and to find the best combinations of variables to maximize the production of antimicrobial compounds (Kheiralla *et al.*, 2018; Guo *et al*., 2018).

In the present research the authors carried out the experiments, to improve antimicrobial metabolite production by *Fusarium falciforme* MNP1 isolated from the rhizosphere soil of *Epipremnum aureum*. OFAT and RSM designs were used to optimize the fermentation conditions.

2. Material and methods

2.1 collection of soil sample

Soil sample from the 15- 20 cm depth of rhizosphere of *Epipremnum aureum* (money plant) was collected in the sterile bags. In the laboratory, soil sample was air-dried at room temperature and pulverised properly. The collected sample was processed within 24 hours.

2.2 Isolation of antimicrobial compound producing fungi

Serial dilution method and pour plate method were used to isolate the antimicrobial compound producing fungal isolate. In the aseptic conditions (laminar air flow chamber), the soil sample was serially diluted. Serial dilutions were prepared up to 10^{-5} and 0.1 ml of each dilution was spread in the blank autoclaved petri plates. After addition of soil sample, the lukewarm Potato Dextrose Agar (PDA) having antibacterial agent (50 µg/ml streptomycin) was poured in the pre-inoculated petri plates. Plates were incubated at the 28˚C in the incubator for5- 7 days. Morphologically different colonies were separated by inoculating each colony on the different plate to maintain the pure culture.

2.3 Test microorganisms

The test microorganisms responsible for causing opportunistic infections were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, which included gram-positive bacteria, *Bacillus subtilis* (MTCC 2274), *Staphylococcus aureus* (MTCC 6908); gram-negative bacteria, *Pseudomonas aeruginosa* (MTCC 647), *Escherichia coli* (MTCC 40) and yeasts namely *Candida albicans* (MTCC 183) and Mold namely *Aspergillus niger* (MTCC 281). All slants were kept at 4°C in a refrigerator for further studies (Awad *et al*., 2023).

2.4 Antimicrobial screening

Agar well diffusion method was used to screen for antimicrobial activity of *Fusarium*. Autoclaved Potato Dextrose Broth (PDB) (100 ml in 250 ml flask) was inoculated with 1-2 fungal discs from 4-5 days old fungal plate. The inoculated flask was placed in incubator at 28

°C for 7- 9 days. After proper sporulation, the fermentation broth was filtered with Whatman no. 1 filter paper and filtrate was used for the antimicrobial screening.

2.5 Morphological and microscopic identification of fungal isolate

Morphologically, fungal isolate was identified based on colony morphology, pigmentation, growth pattern and sporulating structure by lactophenol cotton blue staining by following various manuals and monographs (Gilman, 1967; Job *et al*., 2015). Molecular identification was performed by MTCC CSIR- IMTECH (Chandigarh).

2.4 Optimization of culture conditions for antimicrobial production through OFAT and RSM design

One-factor-at-a-time experimental design

The main culture conditions which affect antimicrobial substance synthesis are fermentation pH, temperature, inoculum size, incubation period, and fermentation vessel size. The range of the factors which were considered in this study, are as follows, pH (4, 5, 6, 7, 8). Temperature (20℃, 25℃, 30℃, 35℃, 40℃), inoculum size (0.25%, 0.5%, 0.75%, 1.0%, 1.25%), fermentation vessel size (250 ml, 500 ml, 1000 ml, 2000 ml) and incubation period (7 days, 9 days, 11 days, 13 days, 15 days). All the experiments were performed in the triplicates. Standard deviation and mean values were calculated in this model.

Response surface methodology (RSM)

Response surface methodology (RSM) is a mathematical and statistical technique used for optimizing various process parameters and relationship between the variables (response) and independent (various inputs) factors (Mathivanan *et al.*, 2024). A **Central composite design** (CCD) is a statistical model in Response Surface Methodology to obtain a second order (quadratic) equation for the response variable.

In the current study, the Response Surface Methodology (RSM) was employed to identify the effect of cultural conditions of *Fusarium* isolate for higher production of antimicrobial metabolites. Using Origin 13.0.5.0 software with three factors and five levels, the combinations of 20 experiments were generated. Three components pH, temperature (°C) and incubation period (days) were considered for this model. The range of the values considered in the current study as shown in table 1.

Table 1: Fermentation parameters used for central composite design parameters

Factor	Name	Units	-α value	+α value	Low	High	Centre
X_1	Temperature	℃	8.18	41.82	15.00	35.00	25.00
X_2	pH	pH	2.64	9.36	4.00	8.00	6.00
X_3	Incubation period	Days	4.27	17.73	7.00	15.00	11.00

A second order polynomial regression equation with specific coded values was created for the synthesis of antimicrobial metabolites by the fungal isolate in accordance with the Response Surface Methodology Central Composite Design (RSM-CCD) design. After performing the designed experiments, the responses of each run were fitted to independent second order polynomial equation (1):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta ixi \; + \; \sum_{i<j}^{n} \beta ijxixj + \; \sum_{i=1}^{n} \beta jjx2 \qquad \text{- (1)}$$

where Y is the predicted response; three variables were considered in the current study, hence, value 3 was taken for n. Thus, by substituting the value 3 for n, Eq. 1 becomes:

$$Y=\beta_0 + \beta_1x_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{32} + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 \qquad \text{- (2)}$$

where β_0 is an intercept term; X_1, X_2 and X_3 are input variables (viz., temperature, time, and concentration), which affect the response Y; β_1, β_2, and β_3 are linear coefficients; β_{12}, β_{13}, and β_{23} are the interaction coefficients; and β_{11}, β_{22}, and β_{33} are the quadratic coefficients.

Statistical data analysis

Mean and Standard deviation were calculated from the triplicate results of OFAT model. On the basis of preliminary OFAT experiment results, Lower and upper coded levels were determined. RSM- CCD model was performed to identify the significant effect of the fermentation parameters by using Design Expert 13.0.5.0 software. This software generated the set of experiment and analysed the results. Responses for the quadratic model system were evaluated through analysis of variance (ANOVA). Statistical significancy of these equations and evaluation of R^2 were identified by the F-test (Fit-statistics). The significance of independent variables and their interactions was tested by using ANOVA. Results were assessed with various descriptive statistics, including p-value, lack of fit test, and F-test, for the developed quadratic mathematical model concerning the experimental data.

Results and discussion

Isolation and antimicrobial screening of the rhizosphere soil fungi

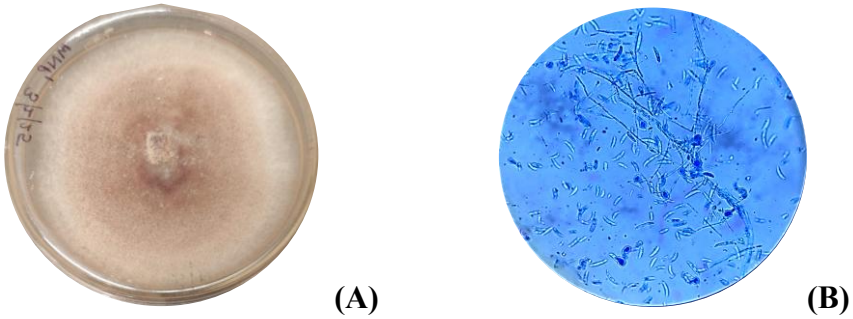
Antimicrobial compound producing fungal isolate was isolated from the rhizosphere of the *Epipremnum aureum* (Money plant), Kurukshetra (INDIA). On the basis of zone of growth inhibition, MNP1 was found to exhibit antimicrobial activity against all the test microorganisms on the basis of zone of growth inhibition.

Morphological and molecular characterisation

Morphologically, *Fusarium* sp. was identified on the basis of sporulation, pigmentation and colony morphology (Fig 1) (Gilman, 1967; Job *et al*., 2015; Bhatnagar and Yadav, 2023). The fungal isolate MNP1 (*Fusarium* sp.) was light brown in colour (white colour boundary) and produced dark reddish brown colour pigment observed from the reverse side of the petri plate. The appearance of the fungal colony was cottony with radial spreading of mycelium growth pattern.

Microscopically, microconidia and macroconidia were observed which are the key feature for identification of *Fusarium* sp. Macroconidia are typically sickle-shaped and microconidia are typically smaller, single-celled, and oval or kidney-shaped, found in chains.

The phylogenetic analysis revealed that MNP1 showed 100% similarity with *Fusarium falciforme* and based on its 18S RNA ITS sequence (Gen Bank accession number PX106850) (Figs. 2).



(A) **(B)**

Figure 1: (A) Morphological and (B) microscopic characterisation of fungal isolate MNP1

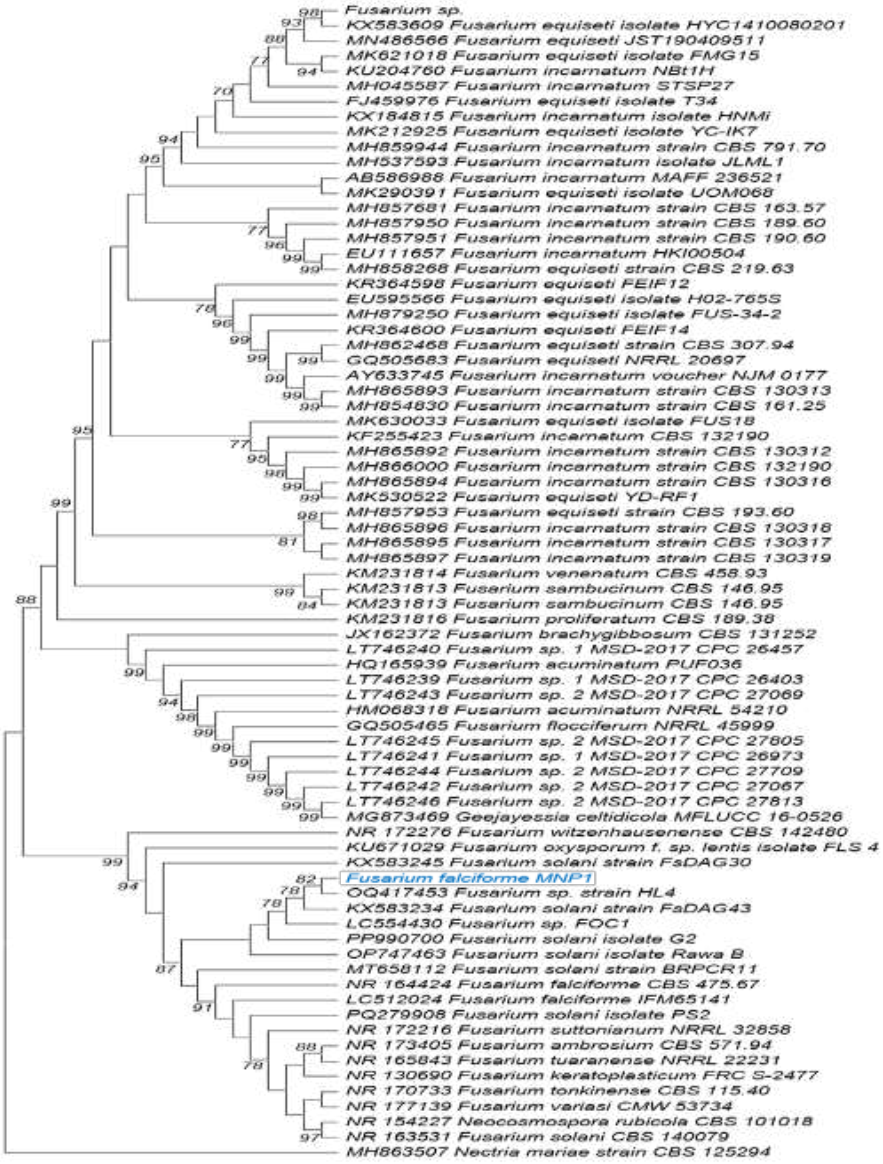


Figure 2: Phylogenetic analysis of *Fusarium falciforme* (MNP1)

Optimization of culture conditions for antimicrobial production through OFAT and RSM design

OFAT design

Antimicrobial compound production by *Fusarium* isolate was found at pH 6 with maximum zone of growth inhibition against all the test microorganisms with 30 mm against *Escherichia coli* (MTCC- 40), 28 mm against *Pseudomonas aeruginosa* (MTCC- 647), 25 mm against *Bacillus subtilis* (MTCC- 2274), 27 mm against *Staphylococcus aureus* (MTCC- 6908), 30 mm against *Aspergillus niger* (MTCC- 281), and 28 mm against *Candida albicans* (MTCC- 183). There was decrease in the zone of growth inhibition with increasing pH values to pH 7 (18 mm

to 26 mm) and pH 8 (14 mm to 26 mm). There was no zone of growth inhibition was obtained at lower pH, that is pH 5 and pH 4, except *Escherichia coli* showed a small zone of growth inhibition at pH 5 (14 mm) (Figure 3, A) . According to the Merlin *et al*. (2013) who optimized the growth and antimicrobial activity of *Fusarium solani*, and found the pH 6 enhanced dry biomass (3.5 mg/ml) and bioactive compound yield (14.72 µg/ml). Moderate amount of biomass and metabolite was produced at pH 5 and pH 7. No growth and antimicrobial production was observed at pH below 3 and above 11.

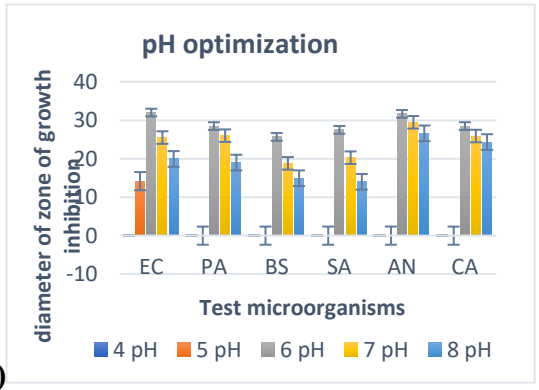
The highest zone of growth inhibition against all the test microorganisms was obtained at 25 ℃ such as 32 mm against *Escherichia coli,* 28 mm against *Pseudomonas aeruginosa*, 25 mm against *Bacillus subtilis*, 28 mm against *Staphylococcus aureus*, 30 mm against *Aspergillus niger*, and 28 mm against *Candida albicans* (Fig 3, B). There was no zone of growth inhibition was obtained at 20℃, 35 ℃ and 40 ℃ against all of the four bacterial test microorganisms. The result of present study was in accordance with study of Gogoi *et al.* (2008), the highest growth (3.2 mg/ml) and synthesis of bioactive metabolites (16.4 µg/ml) by *Fusarium* sp. DF2 were seen at 25°C (±2), followed by 30°C and 35°C, respectively. Both at low temperatures (15°C) and high temperatures (40°C), less growth and the formation of bioactive metabolites were observed.

Incubation period is crucial factor for the production of bioactive metabolites. Incubation period was optimized by growing the fungal isolate at different time durations viz. 7 days, 9 days, 11 days, 13 days, 15 days at 25 ± 2℃, while other parameters were kept constant. The maximum production of antimicrobial compound on the basis of zone of growth inhibition by the *Fusarium falciforme* MNP1 was obtained after 9 days of incubation against bacterial test microorganisms and 15 days incubation against fungal test microorganisms. Maximum zone of growth inhibition after 9 days of incubation was 32 mm against *Escherichia coli*, 25 mm against *Pseudomonas aeruginosa*, 28 mm against *Bacillus subtillis*, and 25 mm against *Staphylococcus aureus* and after 15 days of incubation the zone of growth inhibition was 29 mm against *Candida albicans,* and 33 mm against *Aspergillus niger* (Fig 3, C). The study of Merlin *et al.* (2013) supported the present study that the 9 days incubation period maximize the zone of growth inhibition by enhancing the bioactive metabolite production with *Fusarium sp.*

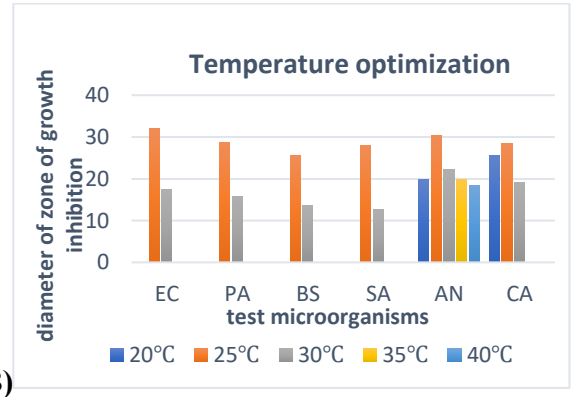
The inoculum concentrations were considered as 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, and 1.25 ml. According to Keshamma *et al.* (2021), 1 ml of spore suspension of inoculum has a final concentration of 1x 107 spores/ml. The inoculum concentrations were considered as 0.25 ml,

0.5 ml, 0.75 ml, 1 ml, and 1.25 ml. On the basis of the maximum zone of growth inhibition, 1 ml of inoculum size was found to be most suitable for the highest production of antimicrobial metabolites. The maximum zone of growth inhibition was obtained at 1 ml spore suspension with 26 mm against *Escherichia coli*, 27 mm against *Pseudomonas aeruginosa*, 20 mm against *Bacillus subtilis*, 22 mm against *Staphylococcus aureus*, 28 mm against *Aspergillus niger*, and 23 mm against *Candida albicans.* The antimicrobial activity of the bioactive metabolites was increased with an increase of spore suspension from 0.25 ml to 1.00 ml, highest at 1.00 ml spore suspension, and at 1.25 ml spore suspension, there was a decrease in the antimicrobial activity of the bioactive metabolites (fig 3, D). In the study by Keshamma *et al.* (2021), out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 ml), 1.0 ml of inoculum was found to be the most suitable for highest production of chitinase by *Fusarium* sp. KS 06, this favours the readings present study of inoculum size.

In the present study, different fermentation vessel sizes viz. 250ml, 500ml, 1000ml, 2000ml were considered. As in 500 ml fermentation vessel, bioactive metabolite showed maximum antimicrobial activity (31 mm against *Escherichia coli*, 30 mm against *Pseudomonas aeruginosa*, 21 mm against *Bacillus subtilis*, 29 mm against *Staphylococcus aureus*, 30 mm against *Aspergillus niger*, and 25 mm against *Candida albicans)* followed by 1000 ml fermentation vessel size (19 mm to 28 mm), 2000 ml fermentation vessel size (12 mm to 21 mm) and 250 ml fermentation vessel size (14 mm to 28 mm) (Figure 3, E). Jain and Pundir (2011) also observed 500 ml fermentation vessel as optimum vessel size to maximize the antimicrobial metabolite production against *Aspergillus terreus,* in terms of zone of growth inhibition.

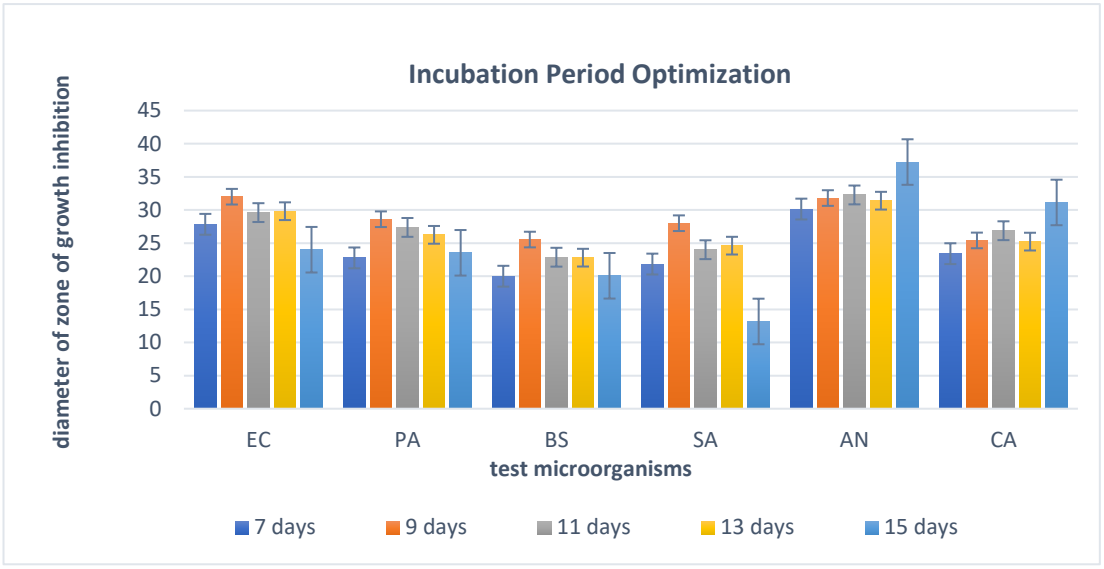


(A)

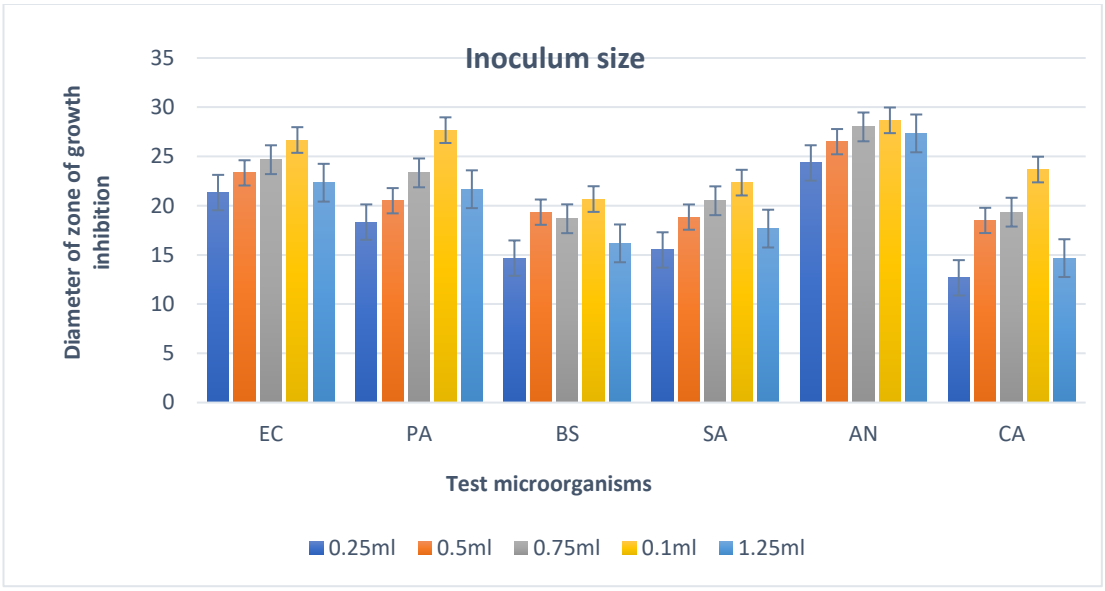


(B)

(C)


Incubation Period Optimization
diameter of zone of growth inhibition
45
40
35
30
25
20
15
10
5
0
EC
PA
BS
SA
AN
CA
test microorganisms
7 days
9 days
11 days
13 days
15 days

(D)


Inoculum size
Diameter of zone of growth inhibition
35
30
25
20
15
10
5
0
EC
PA
BS
SA
AN
CA
Test microorganisms
0.25ml
0.5ml
0.75ml
0.1ml
1.25ml

(E)

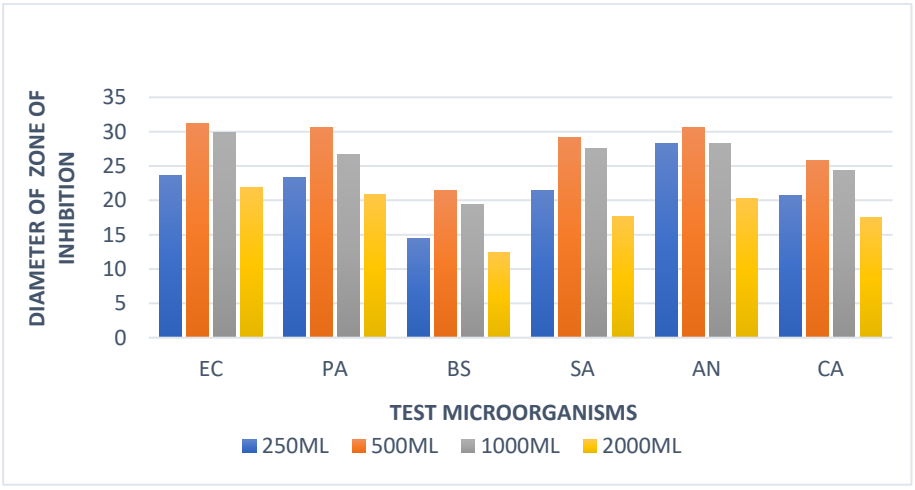

DIAMETER OF ZONE OF INHIBITION
35
30
25
20
15
10
5
0
EC
PA
BS
SA
AN
CA
TEST MICROORGANISMS
250ML
500ML
1000ML
2000ML

Figure 3: Effect of fermentation conditions on the production of antimicrobial metabolite (A) pH; (B) Incubation temperature; (C) Incubation period; (D) Inoculum size; (E) Fermentation vessel size

Response Surface Methodology

To determine the optimum fermentation conditions, a three-factor and three-level CCD model was used to study the interaction between the three factors. The obtained RSM experimental data was fitted to the quadratic model (suggested) for representing the relationship between the factors and response. The cubic model was found aliased, could not be used for further modelling of the experimental data. Aliased model occurs due to lack of experimental run to independently estimate all the terms for that model (Priyadharsini and Dawn, 2023). The result of experiment performed on RSM revealed that the maximum antimicrobial activity was obtained at pH 6, temperature 25 ◦C and an incubation period of 9 days. The experimental results of RSM conclude the significance of the three factors considered in the CCD model for the production of antimicrobial compound. CCD model (Tables 3) represents the predicted and experimentally observed responses (zone of inhibition) of *Fusarium falciforme* MNP1. On the basis of ANOVA analysis, the coefficients of the regression equation were determined, and the readings were fitted to a second order polynomial equation (Urailluck *et al.,* 2015). The following regression equation can be used to express the response of antibacterial activity against all the test microorganisms:

Y (*E. coli*) = $31.0194 - 0.2929A + 1.7574B - 3.1120C - 0.5AB + 0.5AC - 3BC - 11.0867A^2 - 11.0867B^2 - 5.6066C^2$

Y (*P. aeruginosa*) = $28.892 - 0.073A + 1.538B - 4.001C + 2.375AB + 2.625AC - 0.125BC - 9.546A^2 - 9.546B^2 - 6.011C^2$

Y (*B. subtilis*) = $22.473 - 0.732A + 0.732B - 1.964C - 1.25AB + 1.25AC - 1.25BC - 7.776A^2 - 7.776B^2 - 6.008C^2$

Y (*S. aureus*) = $25.002 - 0.732A + 0.732B - 2.703C - 1.25AB + 1.25AC - 1.25BC - 8.852A^2 - 8.852B^2 - 6.023C^2$

Y (*C. albicans*) = $24.002 - 1.538A + 4.380B + 1.221C - 2.625AB + 0.875AC + 1.875BC - 8.499A^2 - 6.731B^2 - 3.372C^2$

Y (*A. niger)* = 34.938 − 0.220A + 6.776B + 1.591C − 0.375AB − 0.125AC + 1.875BC − 11.971A^2 − 9.497B^2 − 5.608C^2

Where Y denotes the antimicrobial activity response, and A, B, C were the coded pH, temperature, and incubation period values, respectively. ANOVA was used to determine the statistical value for the fittness of the model (Table 2) which revealed that the model was significant for *E. coli, P. aeruginosa, B. subtilis, S. aureus*, *C. albicans,* and *A. niger* as the F-values 119.62, 35.07, 63.56, 43.55, 39.60, and 55.13, respectively and the lower 'P' values (<0.0001) obtained with the F-test. Lower p value shown the greater significance (Dabaghi *et al.,* 2023). The coefficient of determination (R^2)values 0.9908, 0.9693, 0.9828, 0.9751, 9727 and 0.9802, for *E. coli, P. aeruginosa, B. subtilis, S. aureus*, *C. albicans,* and *A.niger* respectively were indicating that the model could accurately interaction between observed and predicted values. The adjusted coefficient of determination (R^2) values 0.9825, 0.9417, 0.9674, 0.9725, 9481 and 0.9625 for all responses, were revealed the model's fittness. The R^2 values (close to 1) demonstrated a significant correlation between the predicted and experimental responses. The coefficient of variation (C.V.) for this model was 15.31% (*E. coli*), 26.28% (*P. aeruginosa*), 24.13% (*B. subtilis*), 28.66% (*S. aureus*), 11.29% (*C. albicans*), and 17.17% (*A.niger),* showing reflection of equations for the test values. By this it can be concluded, the regression model could significantly predict the antimicrobial activity of *Fusarium flaciforme* MNP1.

Table 2: ANOVA regression analysis of quadratic polynomial model and significance test for optimization response as zone of inhibition

Response variables	**Source**	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Escherichia coli	Model	3478.17	9	386.46	44.74	< 0.0001	**Significant**
	Residual	34.04	10	3.40			
	Lack of Fit	34.04	5	6.81			
	Pure Error	0.0000	5	0.0000			
	Cor Total	3698.95	19				
Pseudomonas aeruginosa	Model	2593.29	9	288.14	133.01	< 0.0001	**Significant**
	Residual	95.37	10	9.53			
	Lack of Fit	95.37	5	19.07			
	Pure Error	0.0000	5	0.0000			
	Cor Total	3105.75	19				
Bacillus subtilis	Model	2056.44	9	228.49	76.66	< 0.0001	**Significant**

	Residual	34.98	10	3.50			
	Lack of Fit	34.98	5	7.00			
	Pure Error	0.0000	5	0.0000			
	Cor Total	2036.25	19				
Staphylococcus aureus	Model	2493.58	9	277.06	43.55	< 0.0001	**Significant**
	Residual	63.62	10	6.36			
	Lack of Fit	63.62	5	12.72			
	Pure Error	0.0000	5	0.0000			
	Cor Total	2557.20	19				
Candida albicans	Model	2007.86	9	223.10	39.60	< 0.0001	**Significant**
	Residual	56.34	10	5.63			
	Lack of Fit	56.34	5	11.27			
	Pure Error	0.0000	5	0.0000			
	Cor Total	2064.20	19				
Aspergillus niger	**Model**	3959.16	9	439.91	55.13	< 0.0001	**Significant**
	Residual	79.79	10	7.98			
	Lack of Fit	79.79	5	15.96			
	Pure Error	0.0000	5	0.0000			

The significant interactive effects of the variables (incubation time–pH, incubation time-tempera ture, pH–temperature) are represented in 3D plots generated by RSM. Each 3-D intraction and countour plots display the effects of two variables, while third variable kept constant for the antimicrobial production for the response *E. coli* (Fig 6)*, P. aeruginosa* (Fig 7)*, B. subtilis* (Fig 8)*, S. aureus* (Fig 9), *C. albicans* (Fig 10)*,* and *A.niger* (Fig 11). The 3-D response surface plots revealed that pH 6, temperature 25 ◦C, and a 9 day incubation period would enhance antimicrobial metabolite production.

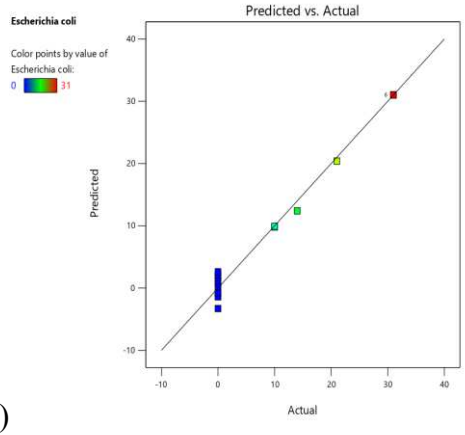
In the study by Zhao *et al.* (2023) to promote the mycelial growth and sporulation of *Fusarium equiseti*, investigated the culture conditions by using BBD (Box Behnken design). As a result of the investigation, an increase of 3.46 and 2.06 times higher mycelial growth and sporulation respectively was recorded.

Another example for importance of RSM was coat by Aliyu *et al.* (2025), used the RSM (CCD model) to enhance the production of carotenoid pigments by *Fusarium oxysporum, Fusarium moniliforme,* and *Fusarium solani.* The variables analysed for the study temperature (25°C–45°C), incubation time (7 days), peptone (0.1–0.3 g/l), fructose (0.1–0.3 g/l), and initial pH (4–8).

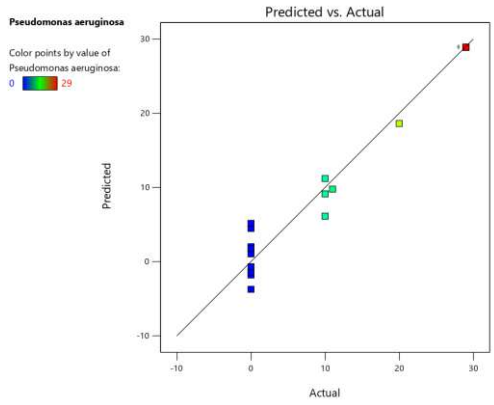
Validation of the results observed

To evaluate the reliability of the RSM results, the experiments at optimal conditions were performed. Three-dimensional (3-D) response surface diagnostic plots were shown the model's acurracy and demonstrate individual and interactive effects of pH, temperature, and incubation period on antimicrobial metabolite production by the *Fusarium falciforeme* MNP1. Actual versus predicted responses are illustrated by the diagnostic plots (fig 5), which were rely within the range of the operating variables.

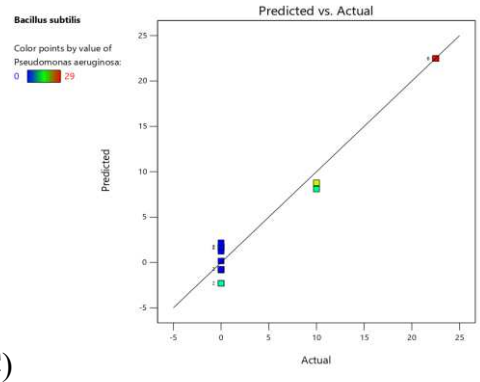
The experimental results illustrated an increase in antimicrobial activity with zone of growth inhibition of 31 mm (*E. coli*), 28 mm (*P. aeruginosa*), 22 mm (*B. subtilis*), 25 mm (*S. aureus*), 34 mm (*A. niger*), and 24 mm (*C. albicans*) which were close to the predicted RSM values (Fig. 4). Therefore, model's validity was confirmed by the strong agrrement in experimental results and predicted results by the RSM. It can be concluded that by increase in antimicrobial activity that RSM is an effective tool for determining the optimal fermentation conditions of individual variables and the maximum response value (Yun et al., 2018).



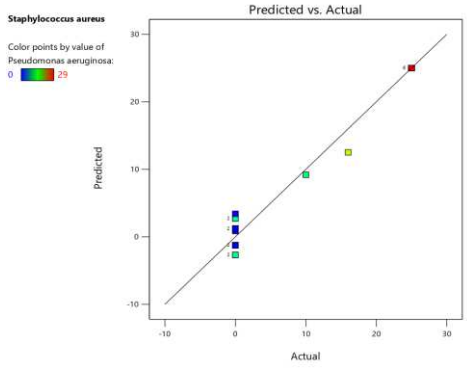
(A)



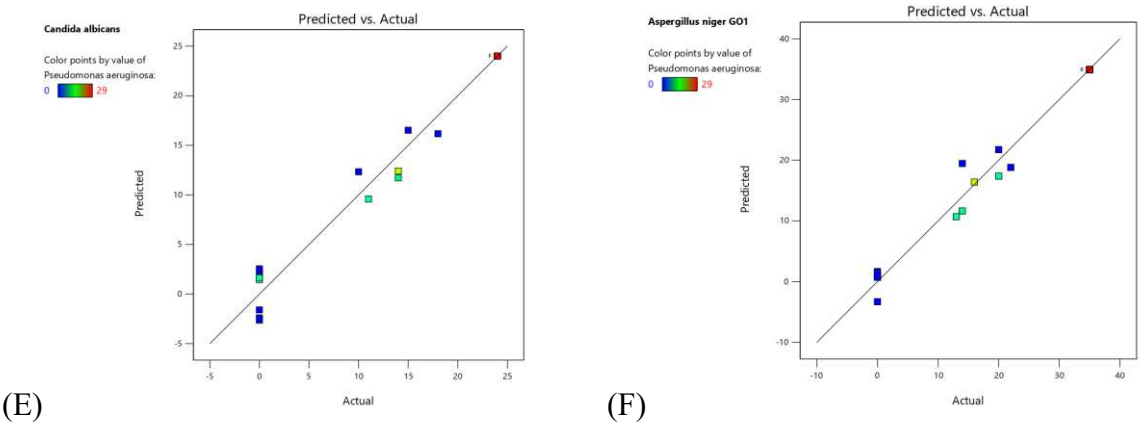
(B)



(C)

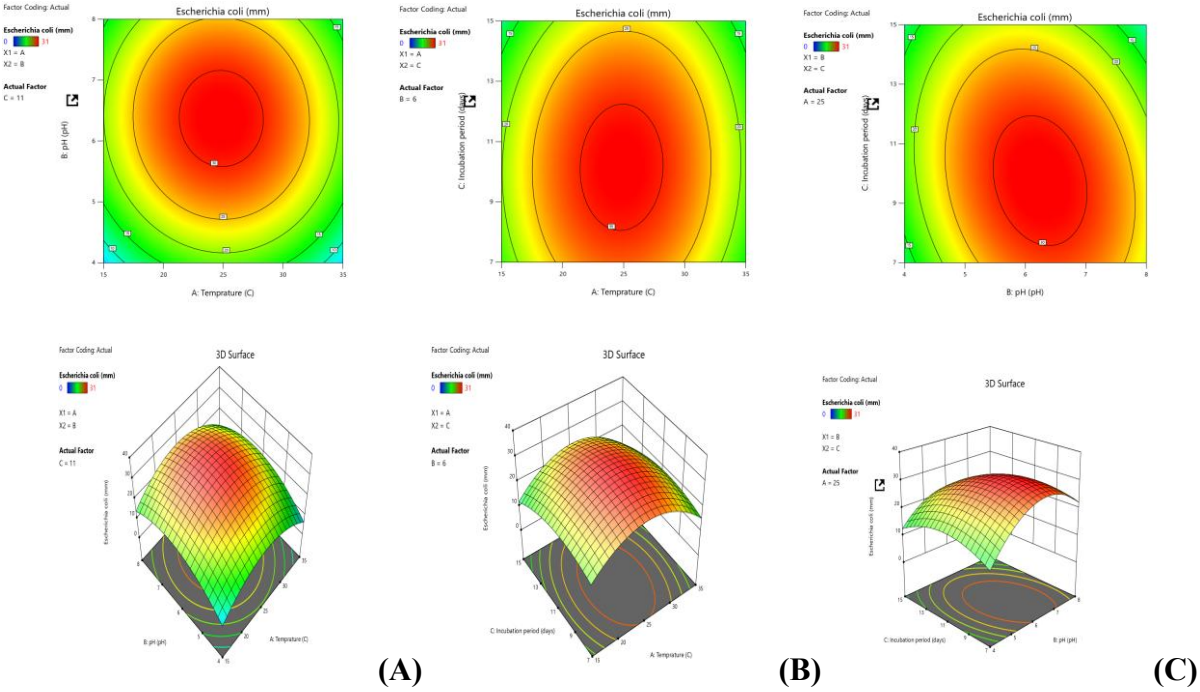


(D)



(E) (F)

Figure 4: Diagnostic plots showing actual versus predicted response (ZOI) against (A) *Escherichia coli*, (B) *Pseudomonas aeruginosa,* (C) *Bacillus subtilis,* (D) *Staphylococcus aureus,* (E) *Candida albicans,* and (F) *Aspergillus niger*



(A) **(B)** **(C)**

Figure 5: Response surface plot and contour plot for *Escherichia coli* showing the effect of (A) temperature and pH, (B)temperature and incubation period, (C) pH and incubation time

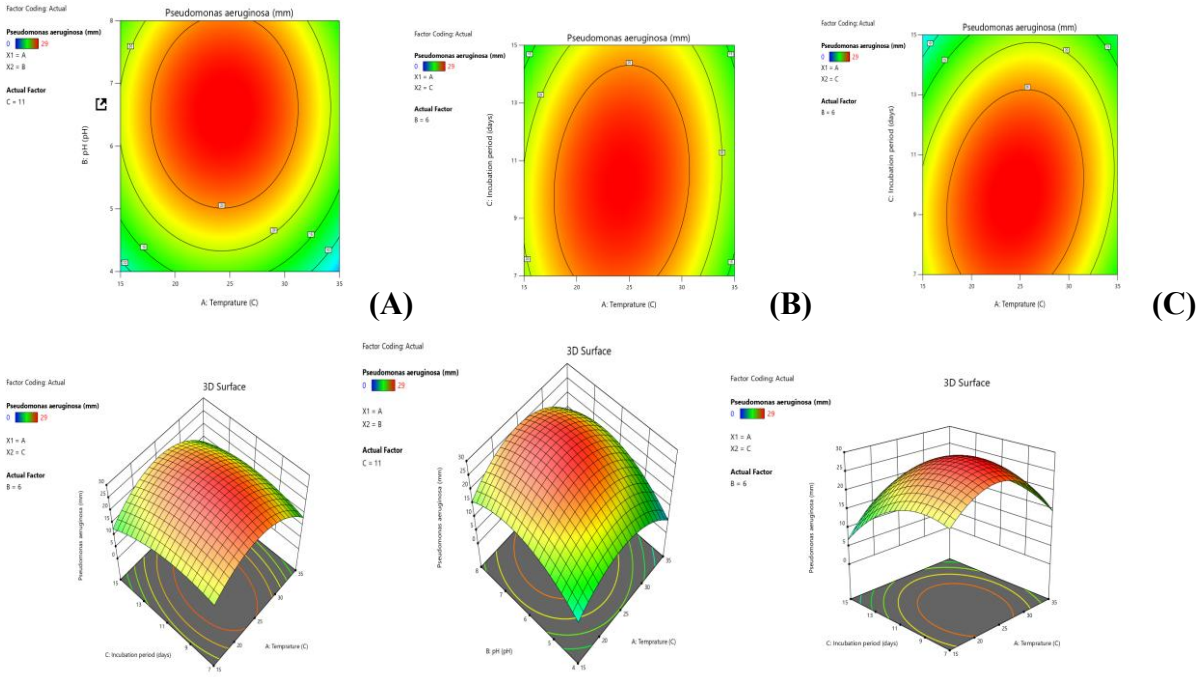


Figure 6: Response surface plot and contour plot for *Pseudomonas aeruginosa* showing the effect of (A) temperature and pH, (B)temperature and incubation period, (C) pH and incubation time

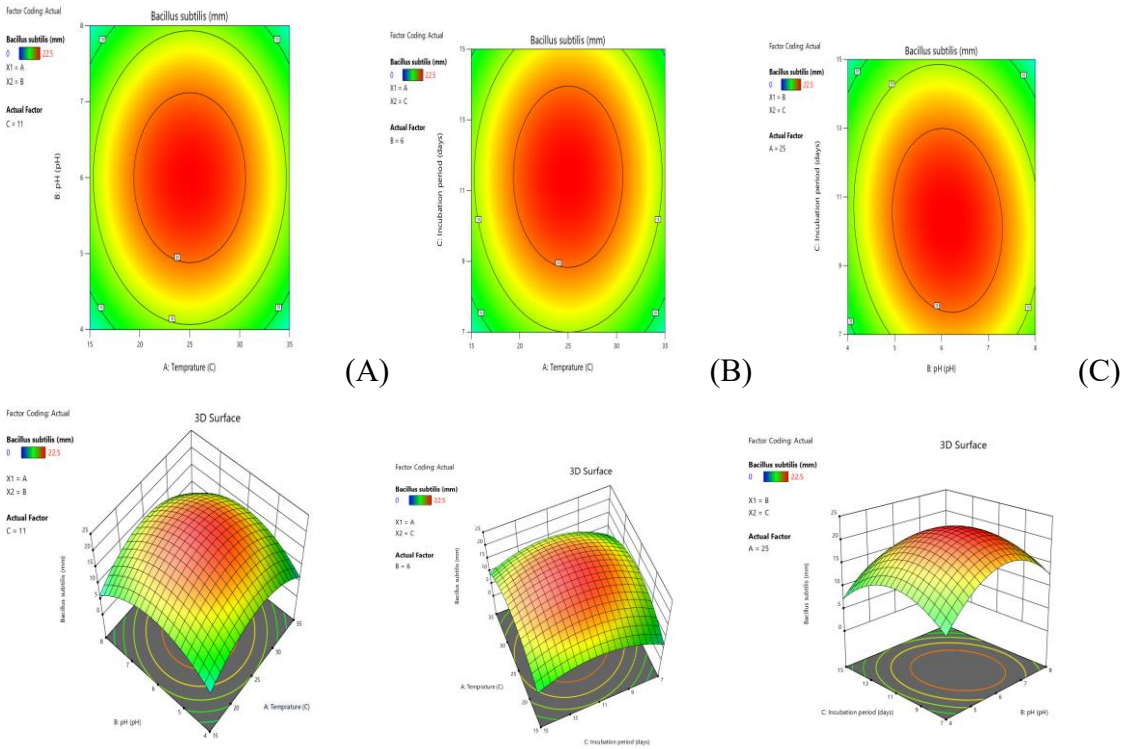


Figure 7: Response surface plot and contour plot for *Bacillus subtilis* showing the effect of (A) temperature and pH, (B)temperature and incubation period, (C) pH and incubation time

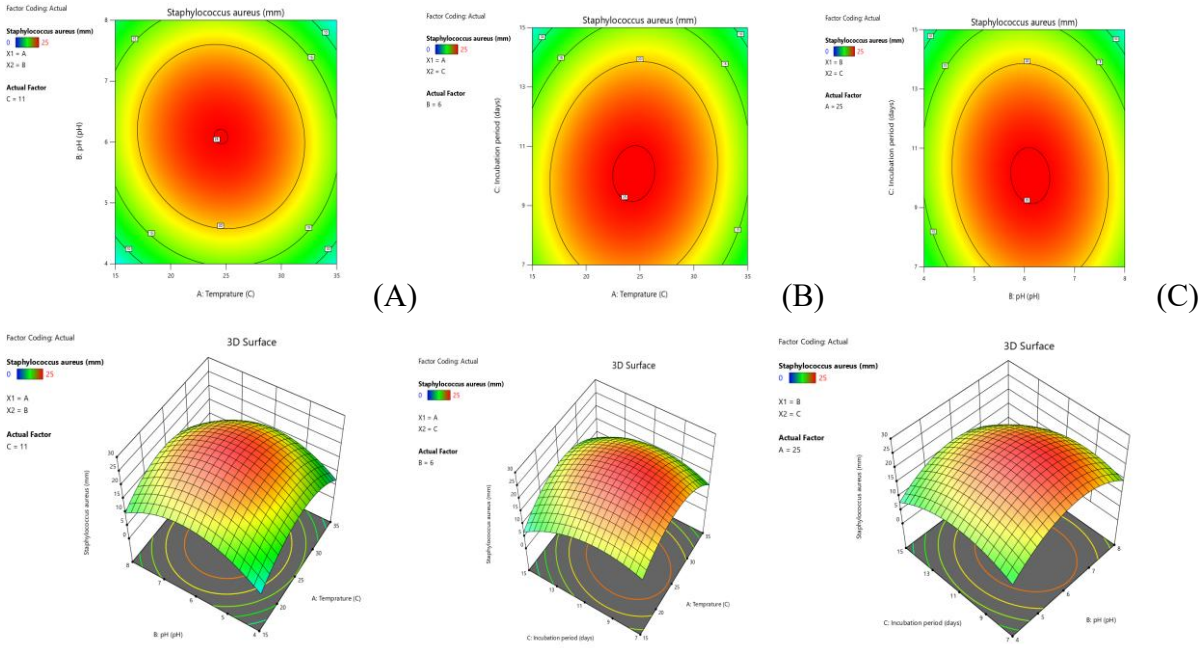


Figure 8: Response surface plot and contour plot for *Staphylococcus aureus* showing the effect of (A) temperature and pH, (B)temperature and incubation period, (C) pH and incubation time

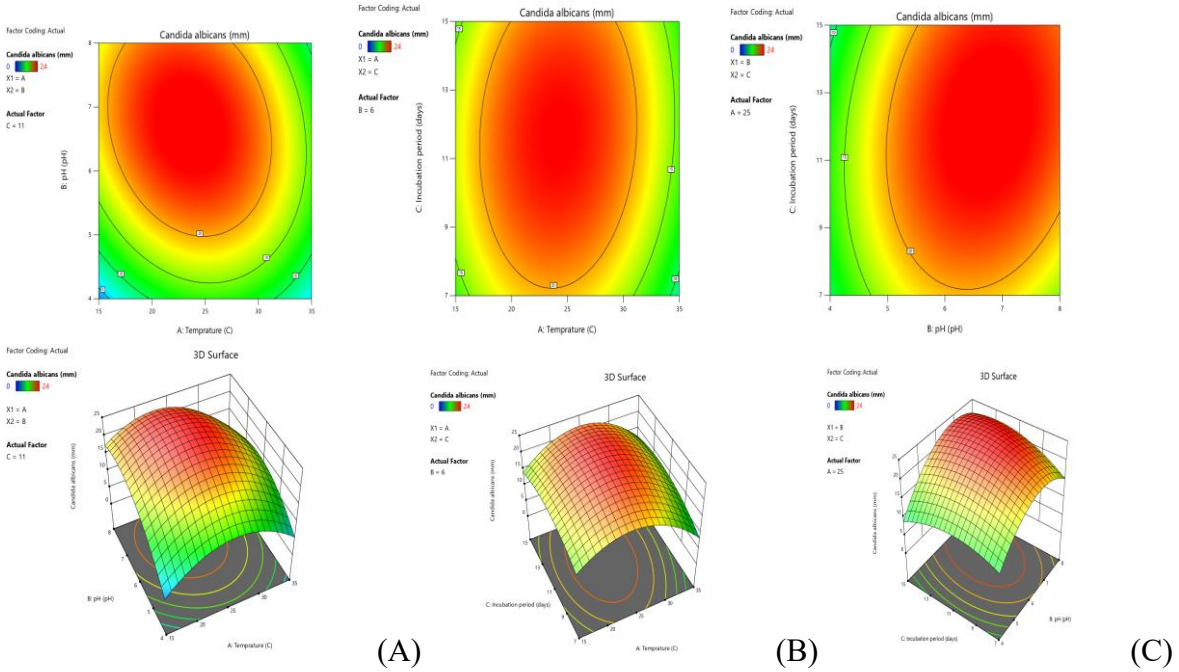


Figure 9: Response surface plot and contour plot for *Candida albicans* showing the effect of (A) temperature and pH, (B)temperature and incubation period, (C) pH and incubation time

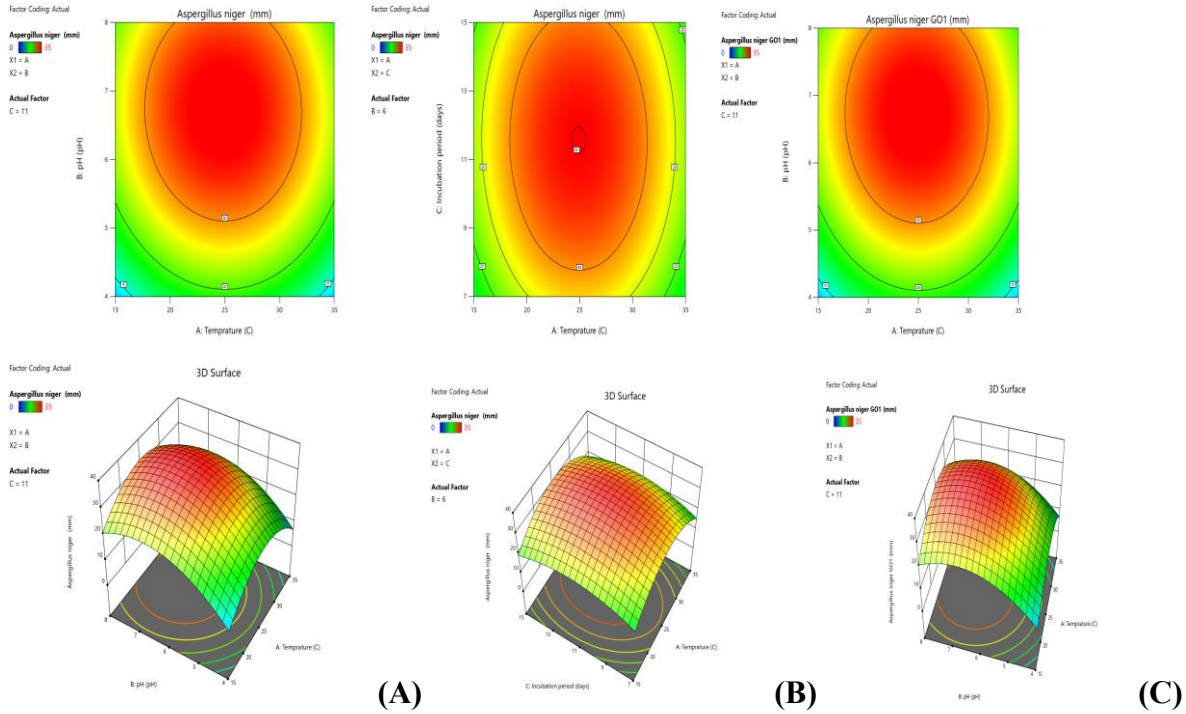


Figure 10: Response surface plot and contour plot for *Aspergillus niger* showing the effect of (A) temperature and pH, (B) temperature and incubation period, (C) pH and incubation time

Table 3: Full factorial central composite design matrix and their observed responses for bioactive metabolite production

Run	Temperature (°C)	pH	Incubation period (days)	*E. coli*		*P. aeruginosa*		*B. subtilis*		*S. aureus*		*C. albicans*		*Aspergillus niger*	
				Actual value	RSM	Actual value	RSM	Actual value	RSM	Actual value	RSM	Actual value	RSM	Actual value	RSM
1	15	4	7	0.0000	1.89	10.00	11.20	0.0000	1.46	0.0000	1.09	0.0000	1.46	0.0000	1.09
2	25	6	11	31.00	31.02	29.00	28.89	24.00	24.00	35.00	34.94	24.00	24.00	35.00	34.94
3	25	6	11	31.00	31.02	29.00	28.89	24.00	24.00	35.00	34.94	24.00	24.00	35.00	34.94

4	25	6	11	31.00	31.02	29.00	28.89	24.00	24.00	35.00	34.94	24.00	24.00	35.00	34.94
5	15	4	15	0.0000	0.6629	0.0000	-1.80	0.0000	-1.60	0.0000	0.7719	0.0000	-1.60	0.0000	0.7719
6	25	6	4.272	21.00	20.40	20.00	18.62	14.00	12.41	16.00	16.40	14.00	12.41	16.00	16.40
7	35	4	7	0.0000	1.30	0.0000	1.05	0.0000	1.89	0.0000	1.65	0.0000	1.89	0.0000	1.65
8	25	9.36	11	0.0000	2.62	0.0000	4.48	10.00	12.33	14.00	19.47	10.00	12.33	14.00	19.47
9	25	6	11	31.00	31.02	29.00	28.89	24.00	24.00	35.00	34.94	24.00	24.00	35.00	34.94
10	15	8	15	0.0000	-0.8223	0.0000	-3.73	18.00	16.16	22.00	18.82	18.00	16.16	22.00	18.82
11	15	8	7	14.00	12.40	11.00	9.77	14.00	11.72	14.00	11.64	14.00	11.72	14.00	11.64
12	25	6	11	31.00	31.02	29.00	28.89	24.00	24.00	35.00	34.94	24.00	24.00	35.00	34.94
13	25	6	11	31.00	31.02	29.00	28.89	24.00	24.00	35.00	34.94	24.00	24.00	35.00	34.94
14	25	6	17.72	10.00	9.93	0.0000	5.16	15.00	16.52	20.00	21.75	15.00	16.52	20.00	21.75
15	35	8	7	10.00	9.82	10.00	9.13	0.0000	1.65	13.00	10.70	0.0000	1.65	13.00	10.70
16	35	4	15	0.0000	2.08	0.0000	-1.45	0.0000	2.33	0.0000	0.8326	0.0000	2.33	0.0000	0.8326

17	8.182	6	11	0.00000	0.1541	0.00000	2.01	0.00000	2.55	0.00000	1.45	0.00000	2.55	0.00000	1.45
18	41.817	6	11	0.00000	-0.8311	0.00000	1.77	0.00000	-2.62	0.00000	0.7085	0.00000	-2.62	0.00000	0.7085
19	35	8	15	0.00000	-1.41	10.00	6.13	11.00	9.59	20.00	17.39	11.00	9.59	20.00	17.39
20	25	2.636641	11	0.00000	-3.29	0.00000	-0.695	0.00000	-2.40	0.00000	-3.32	0.00000	-2.40	0.00000	-3.32

Conclusion

In conclusion, rhizosphere is the hotspot for the microorganisms which have ability to synthesis the various bioactive compounds. *Fusarium* genera are responsible for synthesis of vast number of bioactive metabolites. In the present study, the antimicrobial metabolite produced by the *Fusarium falciforme* MNP1 exhibited the potent antibacterial and antifungal activity against all the test microorganisms. Optimization of the fermentation conditions by using OFAT and RSM indicated the maximum production of antimicrobial compounds by the fungal isolate at pH 6 and incubation at 25 ◦ C for 9 days.

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Declarations

Ethical approval: This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed consent: All the authors have their consent.

Conflicts of interest: The authors declare no conflict of interest.

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